

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
25 May 2001 (25.05.2001)

PCT

(10) International Publication Number  
**WO 01/36635 A2**

(51) International Patent Classification<sup>7</sup>: **C12N 15/12**,  
C07K 14/475, G01N 33/53, C12Q 1/68, A61K 38/18,  
31/70, 39/395, A01K 67/027

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(21) International Application Number: PCT/US00/31170

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(22) International Filing Date:  
15 November 2000 (15.11.2000)

(25) Filing Language: English

(26) Publication Language: English

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(30) Priority Data:  
60/165,733 15 November 1999 (15.11.1999) US  
60/166,178 18 November 1999 (18.11.1999) US  
60/166,288 18 November 1999 (18.11.1999) US  
60/166,143 18 November 1999 (18.11.1999) US  
60/167,471 24 November 1999 (24.11.1999) US  
Not furnished 14 November 2000 (14.11.2000) US

(81) Designated States (national): AE, AG, AL, AM, AT, AU,  
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ,  
DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,  
HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,  
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ,  
NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,  
TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(63) Related by continuation (CON) or continuation-in-part  
(CIP) to earlier applications:

US	60/165,733 (CIP)
Filed on	15 November 1999 (15.11.1999)
US	60/166,143 (CIP)
Filed on	18 November 1999 (18.11.1999)
US	60/166,288 (CIP)
Filed on	18 November 1999 (18.11.1999)
US	60/166,178 (CIP)
Filed on	18 November 1999 (18.11.1999)
US	60/167,471 (CIP)
Filed on	24 November 1999 (24.11.1999)
US	Not furnished (CIP)
Filed on	14 November 2000 (14.11.2000)

(84) Designated States (regional): ARIPO patent (GH, GM,  
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian  
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European  
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,  
IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF,  
CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

**Published:**  
— Without international search report and to be republished  
upon receipt of that report.

For two-letter codes and other abbreviations, refer to the "Guid-  
ance Notes on Codes and Abbreviations" appearing at the begin-  
ning of each regular issue of the PCT Gazette.

(54) Title: NOVEL GROWTH FACTOR POLYPEPTIDES AND NUCLEIC ACIDS ENCODING SAME

(57) Abstract: The present invention provides FCTRX, a novel isolated polypeptide, as well as a polynucleotide encoding FCTRX and antibodies that immunospecifically bind to FCTRX or any derivative, variant, mutant, or fragment of the FCTRX polypeptide, polynucleotide or antibody. The invention additionally provides methods in which the FCTRX polypeptide, polynucleotide and antibody are used in detection and treatment of a broad range of pathological states, as well as to other uses.

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## **NOVEL GROWTH FACTOR POLYPEPTIDES AND NUCLEIC ACIDS ENCODING SAME**

### **BACKGROUND OF THE INVENTION**

The invention generally relates to nucleic acids and polypeptides encoded therefrom. More specifically, the invention relates to nucleic acids encoding membrane bound and secreted polypeptides, as well as vectors, host cells, antibodies, and recombinant methods for producing these nucleic acids and polypeptides.

### **SUMMARY OF THE INVENTION**

The invention is based, in part, upon the discovery of a novel polynucleotide sequences encoding novel polypeptides.

Accordingly, in one aspect, the invention provides an isolated nucleic acid molecule that includes the sequence of SEQ ID NO: 1, 3, 5, 7, 9 or 11 or a fragment, homolog, analog or derivative thereof. The nucleic acid can include, *e.g.*, a nucleic acid sequence encoding a polypeptide at least 85% identical to a polypeptide that includes the amino acid sequences of SEQ ID NO: 2, 4, 6, 8, 10 or 12. The nucleic acid can be, *e.g.*, a genomic DNA fragment, or a cDNA molecule.

Also included in the invention is a vector containing one or more of the nucleic acids described herein, and a cell containing the vectors or nucleic acids described herein.

The invention is also directed to host cells transformed with a vector comprising any of the nucleic acid molecules described above.

In another aspect, the invention includes a pharmaceutical composition that includes an FCTR<sub>X</sub> nucleic acid and a pharmaceutically acceptable carrier or diluent.

In a further aspect, the invention includes a substantially purified FCTR<sub>X</sub> polypeptide, *e.g.*, any of the FCTR<sub>X</sub> polypeptides encoded by an FCTR<sub>X</sub> nucleic acid, and fragments, homologs, analogs, and derivatives thereof. The invention also includes a pharmaceutical composition that includes an FCTR<sub>X</sub> polypeptide and a pharmaceutically acceptable carrier or diluent.

In still a further aspect, the invention provides an antibody that binds specifically to an FCTR<sub>X</sub> polypeptide. The antibody can be, *e.g.*, a monoclonal or polyclonal antibody, and fragments, homologs, analogs, and derivatives thereof. The invention also includes a pharmaceutical composition including FCTR<sub>X</sub> antibody and a pharmaceutically acceptable

carrier or diluent. The invention is also directed to isolated antibodies that bind to an epitope on a polypeptide encoded by any of the nucleic acid molecules described above.

The invention also includes kits comprising any of the pharmaceutical compositions described above.

The invention further provides a method for producing an FCTR<sub>X</sub> polypeptide by providing a cell containing an FCTR<sub>X</sub> nucleic acid, *e.g.*, a vector that includes an FCTR<sub>X</sub> nucleic acid, and culturing the cell under conditions sufficient to express the FCTR<sub>X</sub> polypeptide encoded by the nucleic acid. The expressed FCTR<sub>X</sub> polypeptide is then recovered from the cell. Preferably, the cell produces little or no endogenous FCTR<sub>X</sub> polypeptide. The cell can be, *e.g.*, a prokaryotic cell or eukaryotic cell.

The invention is also directed to methods of identifying an FCTR<sub>X</sub> polypeptide or nucleic acid in a sample by contacting the sample with a compound that specifically binds to the polypeptide or nucleic acid, and detecting complex formation, if present.

The invention further provides methods of identifying a compound that modulates the activity of an FCTR<sub>X</sub> polypeptide by contacting an FCTR<sub>X</sub> polypeptide with a compound and determining whether the FCTR<sub>X</sub> polypeptide activity is modified.

The invention is also directed to compounds that modulate FCTR<sub>X</sub> polypeptide activity identified by contacting an FCTR<sub>X</sub> polypeptide with the compound and determining whether the compound modifies activity of the FCTR<sub>X</sub> polypeptide, binds to the FCTR<sub>X</sub> polypeptide, or binds to a nucleic acid molecule encoding an FCTR<sub>X</sub> polypeptide.

In another aspect, the invention provides a method of determining the presence of or predisposition of an FCTR<sub>X</sub>-associated disorder in a subject. The method includes providing a sample from the subject and measuring the amount of FCTR<sub>X</sub> polypeptide in the subject sample. The amount of FCTR<sub>X</sub> polypeptide in the subject sample is then compared to the amount of FCTR<sub>X</sub> polypeptide in a control sample. An alteration in the amount of FCTR<sub>X</sub> polypeptide in the subject protein sample relative to the amount of FCTR<sub>X</sub> polypeptide in the control protein sample indicates the subject has a tissue proliferation-associated condition. A control sample is preferably taken from a matched individual, *i.e.*, an individual of similar age, sex, or other general condition but who is not suspected of having a tissue proliferation-associated condition. Alternatively, the control sample may be taken from the subject at a time when the subject is not suspected of having a tissue proliferation-associated disorder. In some embodiments, the FCTR<sub>X</sub> is detected using an FCTR<sub>X</sub> antibody.

In a further aspect, the invention provides a method of determining the presence of or predisposition of an FCTR<sub>X</sub>-associated disorder in a subject. The method includes providing

a nucleic acid sample, *e.g.*, RNA or DNA, or both, from the subject and measuring the amount of the FCTR<sub>X</sub> nucleic acid in the subject nucleic acid sample. The amount of FCTR<sub>X</sub> nucleic acid sample in the subject nucleic acid is then compared to the amount of an FCTR<sub>X</sub> nucleic acid in a control sample. An alteration in the amount of FCTR<sub>X</sub> nucleic acid in the sample relative to the amount of FCTR<sub>X</sub> in the control sample indicates the subject has a tissue proliferation-associated disorder.

In a still further aspect, the invention provides a method of treating or preventing or delaying an FCTR<sub>X</sub>-associated disorder. The method includes administering to a subject in which such treatment or prevention or delay is desired an FCTR<sub>X</sub> nucleic acid, an FCTR<sub>X</sub> polypeptide, or an FCTR<sub>X</sub> antibody in an amount sufficient to treat, prevent, or delay a tissue proliferation-associated disorder in the subject.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

## DETAILED DESCRIPTION OF THE INVENTION

The invention is based in part on the discovery of novel nucleic acid sequences encoding polypeptides similar to growth factors. The novel nucleic acids described herein and their encoded polypeptides are referred to collectively as "FCTR<sub>X</sub>".

### **FCTR1: Neurite Outgrowth-Promoting Protein Homolog**

Included in the invention is FCTR1, a nucleic acid encoding a polypeptide bearing sequence similarity to neurite outgrowth-promoting protein (also described as "AC009647-A"). Antibodies that bind specifically to FCTR1 polypeptides, and fragments thereof, are included in the invention. The invention further includes fragments, homologs, analogs, and derivatives of FCTR1 nucleic acids, polypeptides, and antibodies.

A neurite outgrowth-promoting protein homolog according to the invention, FCTR1 is related to Neurite Growth-Promoting Factor 2 (NEGF2). NEGF2 is a member of a highly conserved, developmentally regulated human gene family. The gene product exhibits neurite outgrowth-promoting activity and may play a role in nervous system development and/or maintenance. Expression of NEGF2 is thought to be predominant only for a short period from approximately one-half to two-thirds of the way through gestation; before and after that period, it is barely detectable. The gene codes for a 143-residue protein that is a precursor for a mature protein of 121 amino acids and is 46% homologous with another heparin-binding neurite outgrowth-promoting factor, NEGF1, which maps to 7q22-qter. See, e.g., Eddy *et al.*, 1991 (Abstract) *Cytogenet. Cell Genet.* 58: 1920.

NGF1, also called pleiotrophin, is discussed in Online Mendelian Inheritance in Man (OMIM) accession number 162095. NGF2 is discussed in Online Mendelian Inheritance in Man (OMIM) accession number 162096. NEGF2 is also known as midkine (Mdk). Midkine was first found in differentiating mouse teratocarcinoma cells. It has neurotrophic activities and is mitogenic to certain fibroblast cell lines, but not to all fibroblast cell lines.

By study of somatic cell hybrids and cell hybrids carrying translocations involving chromosome 11, it was demonstrated that the NEGF2 gene mapped regionally to 11p13-p11. See, e.g., Eddy *et al.* (1991) above. The Mdk gene was mapped to human chromosome 11p11.2 by fluorescence *in situ* hybridization. See, e.g., Kaname *et al.* 1993 *Genomics* 17: 514-515. The gene for midkine was mapped to mouse chromosome 2 using an interspecific backcross panel and microsatellite polymorphisms as markers. See, e.g., Simon-Chazottes *et al.* 1992 *Mamm Genome* 2: 269-271. Using somatic cell hybrid analysis and interspecific backcross analysis, human Mdk to 11p13-p11 and mouse Mdk, respectively, were mapped to a syntenic region of mouse chromosome 2. O'Hara *et al.* 1995 *Cytogenet Cell Genet* 69: 40-43. A pseudogene of Mdk was also mapped to mouse chromosome 11. O'Hara *et al.* (1995), above.

A nucleic acid of the invention ("AC009647-A") encoding neurite outgrowth-promoting protein homolog and originating from chromosome 11 is shown in TABLE 1A at SEQ ID NO:1. The novel nucleic acid has 434 nucleotides. An open reading frame was identified beginning with an ATG initiation codon at nucleotides 1-3 and ending with a tag codon at nucleotides 430-432. A putative untranslated region following the termination codon is underlined, and the start and stop codons are in bold letters.



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Query:   241 GGAGCCGACTGCAAGTACAAGTTTAAAAAATGGGGTGAGTGTGATGGGGGCACAGGCACC 300
          ||||||||||||||||||||| || ||||||| |||||||||||||||||||||
Sbjct:   266 GGAGCCGACTGCAAGTACAAGTTTGAGAACTGGGGTGCGTGTGATGGGGGCACAGGCACC 325

Query:   301 AAAGTCCGCCAAGGCACCCTGAAGAAGGCGCGCTACAATGCTCAGTGCCAGGAGACCATC 360
          |||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct:   326 AAAGTCCGCCAAGGCACCCTGAAGAAGGCGCGCTACAATGCTCAGTGCCAGGAGACCATC 385

Query:   361 CGGGTCCCCCAGCCCTGCACCCCCAAGACCAAAGCAAAGGCCAAAGCCAAGAAAGGGAAG 420
          || ||| || |||||||||||||||||||||||||||||||||||||||
Sbjct:   386 CGCGTCACCAAGCCCTGCACCCCCAAGACCAAAGCAAAGGCCAAAGCCAAGAAAGGGAAG 445

Query:   421 GGAAAGGACTAGAC 434
          |||||||||||||
Sbjct:   446 GGAAAGGACTAGAC 459

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The encoded amino acid sequence has 137 of 143 amino acid residues (95 %) identical to, and 140 of 143 residues (97 %) positive with, a 143 amino acid residue human midkine precursor (neurite outgrowth-promoting protein) (ptnr: SWISSPROT-ACC:P21741), as shown in TABLE 1D. Numbering of the FCTR1 Query sequence corresponds to the nucleic acid residues that were translated prior to the comparison. Numbering for the P21741 protein corresponds to the polypeptide provided in the Swissprot database. As indicated by the "Expect" value, the probability of this alignment occurring by chance alone is  $4.1e-75$ , an extremely low probability.

**TABLE 1D: BLASTX**

**Query = FCTR1; Sbjct = MIDKINE (SEQ ID NO:17)**

>ptnr:SWISSPROT-ACC:P21741 MIDKINE PRECURSOR (NEURITE OUTGROWTH-PROMOTING PROTEIN) (MK) (MIDGESTATION AND KIDNEY PROTEIN) (AMPHIREGULIN-ASSOCIATED PROTEIN) (ARAP) (NEURITE OUTGROWTH-PROMOTING FACTOR 2) - Homo sapiens (Human), 143 aa.

Plus Strand HSPs:

Score = 762 (268.2 bits), Expect =  $4.1e-75$ , P =  $4.1e-75$

Identities = 137/143 (95%), Positives = 140/143 (97%), Frame = +1

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Query:    1 MQHRGFLLLTLLALLALTSAVAKKKNKVKKGGPGSECAEWAWGPCTPSSKDCGVGFREGT 180
          ||||||||||||||||||||| +|||||||||||||||||||||||||||||||
Sbjct:    1 MQHRGFLLLTLLALLALTSAVAKKKDKVKKGGPGSECAEWAWGPCTPSSKDCGVGFREGT 60

Query:   181 CGAQTQIRICRVPCNWKKEFGADCKYKFKKWGECDDGGTGTKVRQGTLLKKARYNAQCQETI 360
          ||||||||||||||||||||| + || |||||||||||||||||||||||||
Sbjct:    61 CGAQTQIRICRVPCNWKKEFGADCKYKFENWGACDGGTGTKVRQGTLLKKARYNAQCQETI 120

Query:   361 RVPQPCTPKTKAKAKAKKGKGD 429
          || +|||||||||||||||
Sbjct:   121 RVTKPCTPKTKAKAKAKKGKGD 143

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A multiple sequence alignment is given in TABLE 1E, with the FCTR1 protein of the invention shown on line 2, in a ClustalW analysis comparing the protein of the invention

resembling neurite outgrowth-promoting protein with related protein sequences.

MK\_HUMAN (SEQ ID NO:17) is the amino acid sequence from human Neurite Outgrowth-Promoting Factor 2 (Genbank Acc. P21741). MK\_MOUSE (SEQ ID NO:18) is the amino acid sequence from mouse midkine precursor disclosed in (Genbank Acc. P12025).

BAA83783\_Rat (SEQ ID NO:19) is the amino acid sequence from rat midkine (Genbank Acc. BAA83783). MK\_CHICK (SEQ ID NO:20) is the amino acid sequence from chicken midkine (Genbank Acc. P24052). PTA2\_XENLA (SEQ ID NO:21) includes the amino acid sequence from *Xenopus laevis* pleiotrophic factor alpha 2 precursor (Genbank Acc. P48531).

**TABLE 1E: ClustalW alignment of the protein of the invention.**

MK_HUMAN	MQHRGFLLLTLLALLAETSAVAKKKDKVKKGGPGSECAEWAWGPGCTPSSKDCGVGFREG
AC009647_A	MQHRGFLLLTLLALLAETSAVAKKKDKVKKGGPGSECAEWAWGPGCTPSSKDCGVGFREG
MK_MOUSE	MQHRGFLLLTLLALLVWTSAAVAKKKDKVKKG---SECSEWTWGPCTPSSKDCGVGFREG
BAA83783_RAT	MOHRSFLLALVALLAVTTAAVAKKKDKVKKG---SECSEWTWGPCTPSSKDCGVGFREG
MK_CHICK	MOPRGLLLLLALLLAAAEAAAKKKKKKKKEE---SECQDWHWGPCIPNSKDCGLGFREG
PTA2_XENLA	MELRAFCVLLTLLLA VSSQAANKKKKGKGA---SDCTEWTWGSIPNSKDCGASTREG
MK_HUMAN	TCCAQTQRIKCRVPCNWKKEFGADCKYKFENWGACDGGTGTKEVROGTLKKARYNAQCQET
AC009647_A	TCCAQTQRIKCRVPCNWKKEFGADCKYKFENWGACDGGTGTKEVROGTLKKARYNAQCQET
MK_MOUSE	TCCAQTQRIKCRVPCNWKKEFGADCKYKFESWGACDGGTGTKEVROGTLKKARYNAQCQET
BAA83783_RAT	TCCAQTQRIKCRVPCNWKKEFGADCKYKFESWGACDGGTGTKEVROGTLKKARYNAQCQET
MK_CHICK	SCGDESRKFKCKIPCNWKKKFGADCKYKFESWGACDGGTGTKEVROGTLKKARYNAQCQET
PTA2_XENLA	TCKEEIRKGRCKIPCNWKKKFGADCKYKFENWGACDGGTGTKEVROGTLKKARYNAQCQET
MK_HUMAN	IRVTKPCTPRTKAKAKAKKGGKGD
AC009647_A	IRVTPQCTPRTKAKAKAKKGGKGD
MK_MOUSE	IRVTKPCTSKTKSKAKAKKGGKGD
BAA83783_RAT	IRVTKPCTSKTKSKAKAKKGGKGD
MK_CHICK	MYVSKPCTAKMKAKAKAKKGGKGD
PTA2_XENLA	VEAAKPCSLKTKSKSKKGGKGGKGD

Black outlined amino acid residues indicate regions of conserved sequence (*i.e.*, regions that may be required to preserve structural or functional properties); greyed amino acid residues can be mutated to a residue with comparable steric and/or chemical properties without altering protein structure or function (*i.e.* L to V, I, or M); non-highlighted amino acid residues can potentially be mutated to a much broader extent without altering structure or function.

The novel nucleic acid of the invention encoding a protein resembling neurite outgrowth-promoting protein includes the nucleic acid sequence of SEQ ID NO:1, or a fragment thereof. The invention also includes a mutant or variant nucleic acid, any of whose bases may be changed from the bases shown in SEQ ID NO:1, while still encoding a protein that maintains the activities and physiological functions of the protein of the invention resembling neurite outgrowth-promoting protein, or a fragment of such a nucleic acid. The invention further includes nucleic acids whose sequences are complementary to those just described, including nucleic acid fragments that are complementary to any of the nucleic acids just described. The invention additionally includes nucleic acids or nucleic acid fragments, or



complements thereto, whose structures include chemical modifications. Such modifications include, by way of nonlimiting example, modified bases, and nucleic acids whose sugar phosphate backbones are modified or derivatized. These modifications are carried out at least in part to enhance the chemical stability of the modified nucleic acid, such that they may be used, for example, as antisense binding nucleic acids in therapeutic applications in a subject. In the mutant or variant nucleic acids, and their complements, up to 2% or more of the bases may be so changed.

The novel protein of the invention includes proteins resembling the neurite outgrowth-promoting protein whose sequence is provided in SEQ ID NO:2. The invention also includes a mutant or variant protein any of whose residues may be changed from the corresponding residue shown in SEQ ID NO:2 while still encoding a protein that maintains its proteins resembling neurite outgrowth-promoting activities and physiological functions, or a functional fragment thereof. In the mutant or variant protein, up to 5% or more of the residues may be so changed. The invention further encompasses antibodies and antibody fragments, such as  $F_{ab}$  or  $(F_{ab})_2$ , that bind specifically to any of the proteins of the invention.

The nucleic acids and proteins of the invention are useful in potential therapeutic applications implicated in various neurological disorders including neurodegenerative pathologies, and/or in fibroblast growth, tissue repair and wound healing, and finally in conditions such as osteoporosis. For example, a cDNA encoding the proteins resembling neurite outgrowth-promoting protein may be useful in gene therapy. The proteins resembling neurite outgrowth-promoting protein may be useful when they are administered to a subject in need thereof in order to treat pathologies such as neurodegenerative pathologies, or osteoporosis, and in conditions in which there is the need for fibroblast growth, tissue repair or wound healing. The novel nucleic acid encoding proteins resembling neurite outgrowth-promoting protein, and the proteins resembling neurite outgrowth-promoting protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. These materials are further useful in the generation of antibodies that bind specifically to the novel substances of the invention for use in therapeutic or diagnostic methods.

#### **FCTR2: Sperm Antigen He-2 Homolog**

Included in the invention is FCTR2, a nucleic acid encoding a polypeptide bearing sequence similarity to sperm antigen He-2 (also called human epididymal protein 2). FCTR2 is also described as "AF202031-A"). Antibodies that bind specifically to FCTR2 polypeptides, and fragments thereof, are included in the invention. The invention further includes

fragments, homologs, analogs, and derivatives of FCTR2 nucleic acids, polypeptides, and antibodies.

Human epididymal proteins such as He-2 are known to function in sperm maturation. Six human epididymal cDNAs, named HE1-HE6, have been cloned. See, *e.g.*, Kirchhoff *et al.*, 1998 *Andrologia*. 30: 225-232. A porcine homolog of the major secretory protein of human epididymis, HE1, is secreted into the epididymal fluid as a 19-kDa glycoprotein, whose sugar moiety was gradually processed to form a 16-kDa protein during transit through the epididymis. See, Okamura *et al.*, 1999 *Biochim Biophys Acta*. 1438(3):377-387. The HE1 homolog mRNA was detected only in the caput and corpus epididymis among the porcine tissues examined. See, Okamura *et al.* The purified HE1 homolog specifically bound cholesterol with high affinity ( $K_d=2.3$   $\mu$ M), and the binding stoichiometry was determined to be 0.94 mol/mol, suggesting that 1 mol of cholesterol binds to 1 mol of the protein. See, Okamura *et al.* It was also found that the HE1 homolog is a major cholesterol-binding protein in the porcine epididymal fluid, suggesting the possibility that the HE1 homolog is involved in the regulation of the lipid composition of the sperm membranes during the maturation in epididymis. See, Okamura *et al.*

A family of HE2-related complementary DNAs have recently been cloned from human and chimpanzee caput/corpus library. See, *e.g.*, Hamil *et al.*, 2000 *Endocrinology* 141(3):1245-1253 and Frohlich *et al.*, J Androl. 2000 21(3):421-430, respectively. After removal of the leader sequence, each of these proteins is predicted to consist of 1 or 2 out of 4 possible peptide modules, of which two modules have no recognizable homology to known proteins. See, *e.g.*, Frohlich *et al.* The other 2 modules have a distribution of cysteine residues characteristic for beta-defensins, a family of proteins with antimicrobial activity. See, *e.g.*, Frohlich *et al.* He-2 is also believed to be androgen dependent. See, *e.g.*, Hamil *et al.* Therefore, FCTR2 may be used for antimicrobial products and treatments.

In one embodiment, FCTR2 nucleic acid (SEQ ID NO:3) is 352 nucleotides in length and encodes a sperm antigen He-2 homolog. The human FCTR2 nucleic acid sequence is shown in Table 2A. Putative untranslated regions upstream from the start codon and downstream from the termination codon are underlined. Putative start and termination codons are shown in bold.

**TABLE 2A**  
**FCTR2 Nucleotide Sequence (SEQ ID NO:3)**

TGCTCTTGGCAGACATGAGGCAACGATTGCTCCCGTCCGTCACCAGCCTTCTCCTTGTGGCCCTGCTGTTTCCAG  
GATCGTCTCAAGCCAGACATGTGAACCACTCAGCCACTGAGGCTTTCGGAGAACTCAGGGAAAGAGCCCCTGGGC  
AAGGCACAAACGGGTTTCAGCTGCTACGCCACGCAGTGAACGGGACTTCTTACCACCGCGCACCCACCTTACC  
AAGTGCACATCTCTCACCGGGAGGCTCGAGGACCCTCATTTAGGATCTGTGTGGACTTTTAGGGCCTAGATGGG  
CCAGGGGATGTTCCACCGGAATTAGAAATACCATCTGCCATATGCAGCAAG

The encoded FCTR2 protein (SEQ ID NO:4) having 103 amino acid residues is shown in TABLE 2B.

**TABLE 2B**  
**FCTR2 Protein Sequence (SEQ ID NO:4)**

MRQRLPSVTSLLLVALLFPGSSQARHVNHSATEAFGELRERAPGQGTNGFQLLRHAVKRDFLPRTPPYQVHIS  
HREARGPSFRICVDFLGPRWARGCSTGN

Analysis of the first 70 amino acids of AF202031\_A were analysed for signal peptide prediction. The most likely signal peptide cleavage site between amino acid residues 25 and 26, indicated by the dash in the sequence SQA-RH (*i.e.*, SerGlnAla-ArgHis). Thus, FCTR2 protein is most likely an extracellular protein. Embodiments of the invention include alternative forms of FCTR2 either with and without the signal peptide from amino acid positions 1 through 25. For example, in separate embodiments, the invention includes a polypeptide comprising amino acids 26-103 of SEQ ID NO:4, or a polypeptide comprising amino acids 1-103 of SEQ ID NO:4. In a further embodiment, the invention provides a polypeptide comprising amino acids 1-25 of SEQ ID NO:4, or a region of amino acids 1-25 that is sufficient to direct a linked polypeptide sequence to a desired cellular location.

In a search of sequence databases, it was found, for example, that the FCTR2 nucleic acid sequence has 344 of 352 bases identical (97%), and 350 of 352 bases positive, (99%) when aligned in an identity search, to a 673 base pair human He-2 antigen mRNA (SEQ ID NO:22) (GENBANK-ID:HSHE2AA|acc:X67697). As indicated by the "Expect" value, the probability of this alignment occurring by chance alone is 2.1e-71, an extremely low probability. The BLASTN results are shown in TABLE 2C.

**TABLE 2C: BlastN**

**Query = FCTR2; Sbjct = Homo sapiens HE2 (SEQ ID NO:22)**

gb:GENBANK-ID:HSHE2AA|acc:X67697 H.sapiens HE2 mRNA - 673 bp (RNA).

Plus Strand HSPs:

Score = 1718 (257.8 bits), Expect = 2.1e-71, P = 2.1e-71

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Query:      1  TGCTCTTTGGCAGACATGAGGCAACGATTGCTCCCGTCCGTACCAGCCTTCTCCTTGTGG  60
             |||
Sbjct:     85  TGCTCTTTGGCAGACATGAGGCAACGATTGCTCCCGTCCGTACCAGCCTTCTCCTTGTGG  144

Query:     61  CCCTGCTGTTTCCAGGATCGTCTCAAGCCAGACATGTGAACCACTCAGCCACTGAGGCTT  120
             |||
Sbjct:    145  CCCTGCTGTTTCCAGGATCGTCTCAAGCCAGACATGTGAACCACTCAGCCACTGAGGCTC  204

Query:    121  TCGGAGAACTCAGGGAAAGAGCCCCTGGGCAAGGCACAAACGGGTTTCAGCTGCTACGCC  180
             |||
Sbjct:    205  TCGGAGAACTCAGGGAAAGAGCCCCTGGGCAAGGCACAAACGGGTTTCAGCTGCTACGCC  264

Query:    181  ACGCAGTGAAACGGGACTTCTTACCACCGCGCACCCACCTTACCAAGTGACATCTCTC  240
             |||
Sbjct:    265  ACGCAGTGAAACGGGACTTCTTACCACCGCGCACCCACCTTACCAAGTGACATCTCTC  324

Query:    241  ACCGGGAGGCTCGAGGACCCTCATTTAGGATCTGTGTGGACTTTTTAGGGCCTAGATGGG  300
             |||+|||+|||+|||+|||
Sbjct:    325  ACCRGGAGGCTCGAGGACCCTCATTTTARGATCTGYGTGGRCTTTTTAGGGCCTAGATGGG  384

Query:    301  CCAGGGGATGTTCCACCGGGAATTAGAAATACCATCTGCCATATGCAGCAAG  352
             |||+|||+|||
Sbjct:    385  CCAGGGGATGTTCCACYGGGGAATTAGAAATACCATCTGCCRTATGCAGCAAG  436

```

**TABLE 2D: BlastX**

```

Query: 15 MRQRLLPVSTSLLLVALLFPGSSQARHVNHSATEAFGELRERAPGQGTNGFQLLRHAVKR 194
      |||
Sbjct: 1 MRQRLLPVSTSLLLVALLFPGSSQARHVNHSATEALGELRERAPGQGTNGFQLLRHAVKR 60

Query: 195 DFLPPRTPPYQVHISHREARGPSFRICVDFLGPWRARGCSTGN 323
      |
      |||+|||+|||
Sbjct: 61 DLLPPRTPPYQVHISHOEARGPSFKICVGFGLGPWRARGCSTGN 103

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11

(line 2) depicts the human sperm antigen HE2 protein disclosed at Genbank Acc. I37454, HUMAN HE2 PROTEIN -PIR-ID:I37454 (SEQ ID NO:24).

**TABLE 2E: ClustalW Analysis**

PRECURSOR_HU	1	MRQRLPSVTSLLLVALLFPQSSQARHVNHSATEALGELRERAPGQGTNGFQLLRHAVKR
ROTEIN	2	MRQRLPSVTSLLLVALLFPQSSQARHVNHSATEALGELRERAPGQGTNGFQLLRHAVKR
	3	MRQRLPSVTSLLLVALLFPQSSQARHVNHSATEALFELRERAPGQGTNGFQLLRHAVKR
PRECURSOR_HU	1	DLPPRTPPYQVHISHQEARGPSFKICVGFGLGPRWARGCSTGN
ROTEIN	2	DLPPRTPPYQVHISHXEARGPSFXICVXFLGPRWARGCSTGN
	3	DFLPPRTPPYQVHISHREARGPSFRCICVDFLGPRWARGCSTGN

5           Based on the alignment in Table 2E, black outlined amino acids indicate potential regions of conserved sequence (ie structural or functional regions); Greyed amino acids can be mutated to a limited extent without altering protein structure or function (i.e. L to V, I, or M); non-highlighted amino acids can potentially be mutated to a much broader extent without altering structure or function.

10           Possible therapeutic applications for FCTR2 include control of fertility, potential uses toward development of male birth control measures, and anti-sterility applications.

#### **FCTR3 and FCTR4: Somatropin Homologs**

15           Included in the invention are FCTR3 and FCTR4, both somatropin homologs. Antibodies that bind specifically to FCTR3 and FCTR4 polypeptides, and fragments thereof, are included in the invention. The invention further includes fragments, homologs, analogs, and derivatives of FCTR3 and FCTR4 nucleic acids, polypeptides, and antibodies.

20           The somatropin homologs of the invention are two newly disclosed human somatropin variants, which may be splice variants of the same gene. The DNA sequence of FCTR4 shows a deletion of four nucleotides. As a result, the amino acid sequences are the same at the N-terminal end, but differ at the C-terminal end, beginning at the site of translation of the deletion.

25           FCTR3, a somatropin homolog according to the invention, includes the 812 base pair length nucleotide sequence (SEQ ID NO:5) shown in TABLE 3A. Putative untranslated regions preceding the presumed start methionine and following the termination codon are underlined, and the presumed start and termination codons are shown in bold letters.

**TABLE 3A****FCTR3 Nucleotide Sequence (SEQ ID NO:5) (AC013601\_A)**

GCCCAACTCCCCGAACCACTCAGGGTCCTGTGGACAGCTCACCTAGCGGCAATGGCTACAGGCTCCCGGACGTCC  
CTGCTCCTGGCTTTTGGCTGCTCTGCCTGCCCTGGCTTCAAGAGGGCAGTGCCTTCCCAACCATTCCCTTATCC  
AGGCTTTTGGACAACGCTATGCTCCGCGCCCATCGCTGCACCAGCTGGCATATGACACCTACCAGGAGTTTGAA  
GAAGCCTATATCCCAAAGGAACAGAAGTATTTCCTGTCAGAACCCAGACCTCCCTCTGCTTCTCAGAGTCT  
ATTCCAACACCCCTCCAACAGGGAGAAAACACAACAGAAATCCAACCTAGAGCTGCTCCGCATCTCCCTGCTGCTC  
ATCCAGTCATGGCTGGAGCCCGTGCAGCTCCTCAGGAGCGTCTTCGCCAACAGCCTGGTGTACGGCGCCTCTGAC  
AGCAACGTCTATGACCTCCTAAAGGACCTAGAGGAAGGCATCCAAACGCTGATGGGGAGGCTGGAAGATGGCAGC  
CCCCGGAAGTGGGAGATCTTCAAGCAGACCTACAGCAAGTTCGACACAACTCACACAACGATGACGCACTACTC  
AAGAACTACGGGCTGCTCTACTGCTTCAGGAAAGACATGGACAAGGACAAGACATTCTGCGCATCGAGCAATGC  
CGCTCTGTGGAGGGCAGGTGTGGCTTCTAGCTGCCCGGGTGGCATCCCTGTGACCCCTCCCAGTGCCTGTCTG  
GGCCTGGAAGTTGCCACTCCAGTGCCCAACCTGGACCAATAAATTAAACGGGCACATTC

The predicted protein of FCTR3 (SEQ ID NO:6) has 217 amino acid residues, as shown in TABLE 3B:

**TABLE 3B****FCTR3 Protein sequence (SEQ ID NO:6):**

MATGSRTSLLLAFLGLLCLPWLQEGSAFPTIPLSRFLDNAMLRHRLHQLAYDTYQEFEEAYIPKEQKYSFLQNPQ  
 TSLCFSESIPTPSNREKTQQKSNELELLRISLLLIQSWLEPVQLLRSVFANSLVYGASDSNVYDLLKDLEEGIQTL  
 MGRLEDGSPRTGQIFKQTYSKFDTNSHNDALLKNYGLLYCFRKDMDKDKTFLRIEQCRSVEGRCGF

In a search of sequence databases, it was found, for example as shown in TABLE 3C, that the FCTR7 nucleic acid sequence has 777 of 807 bases (96%) identical, and 777 of 807 bases (96%) positive, when aligned in an identity search, to a sequence of a synthetic construct containing human growth factor (SEQ ID NO:25) (Genbank ID E00952, Accession Number E00952). As indicated by the "Expect" value, the probability of this alignment occurring by chance is 7.6e-164, an extremely low likelihood.

**TABLE 3C: BlastN**

**(Query = FCTR3; Sbjct = Human Growth Factor Construct (SEQ ID NO:25))**

gb:GENBANK-ID:E00952|acc:E00952 Plasmid DNA for amplifying human growth factor gene in E.coli - synthetic construct, 3477 bp.

Plus Strand HSPs:

Score = 3749 (562.5 bits), Expect = 7.6e-164, P = 7.6e-164

Identities = 777/807 (96%), Positives = 777/807 (96%), Strand = Plus / Plus

Query: 1 GCCCAACTCCCCGAACCACTCAGGGTCCTGTGGACAGCTCACCTAGCGGCAATGGCTACA 60  
 |||||  
 Sbjct: 304 GCCCAACTCCCCGAACCACTCAGGGTCCTGTGGAC-GCTCACCTAGCTGCAATGGCTACA 362

Query: 61 GGCTCCCGGACGTCCCTGCTCCTGGCTTTTGGCCTGCTCTGCCTGCCCTGGCTTCAAGAG 120  
 |||||  
 Sbjct: 363 GGCTCCCGGACGTCCCTGCTCCTGGCTTTTGGCCTGCTCTGCCTGCCCTGGCTTCAAGAG 422

Query: 121 GGCAGTGCCTTCCCAACCATTCCTTATCCAGGCTTTTGGACAACGCTATGCTCCGCGCC 180

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|||||
Sbjct: 423 GGCAGTGCCTTCCCAACCATTCCTTATCCAGGCTTTTGAACAACGCTAGTCTCCGCGCC 482
Query: 181 CATCGCCTGCACCAGCTGGCATATGACACCTACCAGGAGTTTGAAGAAGCCTATATCCCA 240
|||||
Sbjct: 483 CATCGTCTGCACCAGCTGGCCTTTGACACCTACCAGGAGTTTGAAGAAGCCTATATCCCA 542
Query: 241 AAGGAACAGAAGTATTTCATTCTGCAGAACCCCCAGACCTCCCTCTGCTTCTCAGAGTCT 300
|||||
Sbjct: 543 AAGGAACAGAAGTATTTCATTCTGCAGAACCCCCAGACCTCCCTCTGTTTCTCAGAGTCT 602
Query: 301 ATTCCAACACCCTCCAACAGGGAGAAAACAACAGAAATCCAACCTAGAGCTGCTCCGC 360
|||||
Sbjct: 603 ATTCCGACACCCTCCAACAGGGAGGAAACAACAGAAATCCAACCTAGAGCTGCTCCGC 662
Query: 361 ATCTCCCTGCTGCTCATCCAGTCATGGCTGGAGCCCGTGCAGCTCCTCAGGAGCGTCTTC 420
|||||
Sbjct: 663 ATCTCCCTGCTGCTCATCCAGTCGTGGCTGGAGCCCGTGCAGTTCCTCAGGAGTGTCTTC 722
Query: 421 GCCAACAGCCTGGTGTACGGCGCCTCTGACAGCAACGTCTATGACCTCCTAAAGGACCTA 480
|||||
Sbjct: 723 GCCAACAGCCTGGTGTACGGCGCCTCTGACAGCAACGTCTATGACCTCCTAAAGGACCTA 782
Query: 481 GAGGAAGGCATCCAAACGCTGATGGGGAGGCTGGAAGATGGCAGCCCCGGACTGGGCAG 540
|||||
Sbjct: 783 GAGGAAGGCATCCAAACGCTGATGGGGAGGCTGGAAGATGGCAGCCCCGGACTGGGCAG 842
Query: 541 ATCTTCAAGCAGACCTACAGCAAGTTCGACACAAACTCACACAACGATGACGCACTACTC 600
|||||
Sbjct: 843 ATCTTCAAGCAGACCTACAGCAAGTTCGACACAAACTCACACAACGATGACGCACTACTC 902
Query: 601 AAGAACTACGGGCTGCTCTACTGCTTCAGGAAAGACATGGACAAGGACAAGACATTCTTG 660
|||||
Sbjct: 903 AAGAACTACGGGCTGCTCTACTGCTTCAGGAAGGACATGGACAAGGTCGAGACATTCTTG 962
Query: 661 CGCATCGAGCAATGCCGCTCTGTGGAGGGCAGGTGTGGCTTCTAGCTGCCCGGGTGGCAT 720
|||||
Sbjct: 963 CGCATCGTGCAGTGCCGCTCTGTGGAGGGCAGCTGTGGCTTCTAGCTGCCCGGGTGGCAT 1022
Query: 721 CCCTGTGACCCCTCCCCAGTGCCTGTCTGGGCCTGGAAGTTGCCACTCCAGTGCCACC 780
|||||
Sbjct: 1023 CCCTGTGACCCCTCCCCAGTGCCTGTCTGGGCCTGGAAGTTGCCACTCCAGTGCCACC 1082
Query: 781 AACCCCTGGACCAATAAA-TTAAACGGCA 807
|||||
Sbjct: 1083 AGCCTTGTCCTAATAAAAATTAAGTTGCA 1110

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As shown in TABLE 3D, the full amino acid sequence of the FCTR3 protein was found to have 210 of 217 amino acid residues (96%) identical, and 213 of 217 residues (98%) positive to the 217 amino acid residue human Somatotropin Precursor (SEQ ID NO:26) (ptnr:SWISSNEW - ACC:P01241). As indicated by the "Expect" value, the probability of this alignment occurring by chance alone is 1.1e-109, an extremely low probability.

**TABLE 3D: BlastX**

**(Query = FCTR3; Sbjct = Human Somatotropin Precursor (SEQ ID NO:26))**

ptnr:SWISSNEW-ACC:P01241 SOMATOTROPIN PRECURSOR (GROWTH HORMONE) - Homo sapiens (Human), 217 aa.

Plus Strand HSPs:

Score = 1091 (384.1 bits), Expect = 1.1e-109, P = 1.1e-109

Identities = 210/217 (96%), Positives = 213/217 (98%), Frame = +1

Query: 52 MATGSRTSLLLAFLGLCLPWLQEGSAFPTIPLSRLFDNAMLRAHRLHQLAYDTYQEFEEA 231  
 |||+|||  
 Sbjct: 1 MATGSRTSLLLAFLGLCLPWLQEGSAFPTIPLSRLFDNAMLRAHRLHQLAFDTYQEFEEA 60

Query: 232 YIPKEQKYSFLQNPQTSLCFSES IPTSPNREKTQOKSNLELLRISLLLIQSWLEPVQLLR 411  
 |||+|||  
 Sbjct: 61 YIPKEQKYSFLQNPQTSLCFSES IPTSPNREETQOKSNLELLRISLLLIQSWLEPVQFLR 120

Query: 412 SVFANSLVYGASDSNVYDLLKDLEEGIQTL MGRLEDGSPRTGQIFKQTYSKFDTNSHNDD 591  
 |||  
 Sbjct: 121 SVFANSLVYGASDSNVYDLLKDLEEGIQTL MGRLEDGSPRTGQIFKQTYSKFDTNSHNDD 180

Query: 592 ALLKNYGLLYCFRKDMDDKDTFLRIEQCRSVEGRCGF 702  
 |||+|||  
 Sbjct: 181 ALLKNYGLLYCFRKDMDKVETFLRIVQCRSVEGSCGF 217

TABLE 3E shows a multiple sequence alignment from a ClustalW analysis, with the FCTR3 protein of the invention shown in line 1, denoted as SOMA\_Novel. SOMA\_HUMAN (SEQ ID NO:26) includes the amino acid sequence from (Genbank Acc. P01241). SOMA\_MACMU (SEQ ID NO:27) includes the amino acid sequence from mouse somatotropin precursor (Genbank Acc. P33093). SOMA\_RAT (SEQ ID NO:28) includes the amino acid sequence from rat somatotropin precursor (Genbank Acc. P01244). SOMA\_MOUSE (SEQ ID NO:29) includes the amino acid sequence from mouse somatotropin precursor (Genbank Acc. P06880).

TABLE 3E: ClustalW Analysis

SOMA_Novel	MATGSRTSLLLAFLGLCLPWLQEGSAFPTIPLSRLFDNAMLRAHRLHQLAYDTYQEFEEA
SOMA_HUMAN	MATGSRTSLLLAFLGLCLPWLQEGSAFPTIPLSRLFDNAMLRAHRLHQLAFDTYQEFEEA
SOMA_MACMU	MAAGSRTSLLLAFLGLCLPWLQEGSAFPTIPLSRLFDNAMLRAHRLHQLAFDTYQEFEEA
SOMA_RAT	MAADSQTIPWLLTFSLCLLPWLQEGSAFPTIPLSRLFDNAMLRAHRLHQLAFDTYQEFEEA
SOMA_MOUSE	MATDSRTSWLLTVSLCLLPWLQEGSAFPTIPLSRLFDNAMLRAHRLHQLAFDTYQEFEEA
SOMA_Novel	YIPKEQKYSFLQNPQTSLCFSES IPTSPNREKTQOKSNLELLRISLLLIQSWLEPVQLLR
SOMA_HUMAN	YIPKEQKYSFLQNPQTSLCFSES IPTSPNREETQOKSNLELLRISLLLIQSWLEPVQFLR
SOMA_MACMU	YIPKEQKYSFLQNPQTSLCFSES IPTSPNREETQOKSNLELLRISLLLIQSWLEPVQFLR
SOMA_RAT	YIPEGORYS-ITQNAQAACFSEITIPATIGKEEACQRTDMELLRFSLLLIQSWLCPVQFLS
SOMA_MOUSE	YIPEGORYS-ITQNAQAACFSEITIPATIGKEEACQRTDMELLRFSLLLIQSWLCPVQFLS
SOMA_Novel	SVFANSLVYGASDSNVYDLLKDLEEGIQTL MGRLEDGSPRTGQIFKQTYSKFDTNSHNDD
SOMA_HUMAN	SVFANSLVYGASDSNVYDLLKDLEEGIQTL MGRLEDGSPRTGQIFKQTYSKFDTNSHNDD
SOMA_MACMU	SVFANSLVYGTSYSDVYDLLKDLEEGIQTL MGRLEDGSSRTGQIFKQTYSKFDTNSHNDD
SOMA_RAT	RIFTNLSLMEGTSDR-VYEKLKDLEEGIQALMQELEDGSPRTGQILKQTYDKFDANMRSDD
SOMA_MOUSE	RIFTNLSLMEGTSDR-VYEKLKDLEEGIQALMQELEDGSPRVGQILKQTYDKFDANMRSDD
SOMA_Novel	ALLKNYGLLYCFRKDMDDKDTFLRIEQCR-SVEGRCGF
SOMA_HUMAN	ALLKNYGLLYCFRKDMDKVETFLRIEQCR-SVEGSCGF
SOMA_MACMU	ALLKNYGLLYCFRKDMDDKDTFLRIEQCR-SVEGSCGF
SOMA_RAT	ALLKNYGLLSCFKDKDTHAETLRVMKCRFAESSCAF
SOMA_MOUSE	ALLKNYGLLSCFKDKDTHAETLRVMKCRFVRESSCAF

Black outlined amino acids indicate potential regions of conserved sequence (*i.e.* structural or functional regions); Greyed amino acids can be mutated to a limited extent without altering protein structure or function (*i.e.* L to V, I, or M); non-highlighted amino



acids can potentially be mutated to a much broader extent without altering structure or function.

A second Somatotropin Homolog, FCTR4, includes the FCTR4 nucleotide sequence (SEQ ID NO:7) shown in TABLE 4A. Putative untranslated regions preceding the presumed start methionine and following the termination codon are underlined, and the presumed start and termination codons are shown in bold letters.

**TABLE 4A**  
**FCTR4 Nucleotide Sequence (SEQ ID NO:7) (AC013601\_B)**

**Human Growth Hormone Isoform-V3**

GCCCAACTCCCCGAACCACTCAGGGTCCTGTGGACAGCTCACCTAGCGGCAATGGCTACAGGCTCCCGGACGTCCC  
TGCTCCTGGCTTTTGGCCCTGCCTGCCTGCCCTGGCTTCAAGAGGGCAGTGCCTTCCCAACCATTCCTTATCCAG  
GCTTTTGGACAACGCTATGCTCCGCGCCCATCGCTGCACCACTGGCATATGACACCTACCAGGAGTTTGAAGAA  
GCCTATATCCCAAAGGAACAGAAGTATTCATTCCCTGCAGAACCCCCAGACCTCCCTCTGCTTCTCAGAGTCTATTC  
CAACACCCCTCCAACAGGGAGAAAACACAACAGAAATCCAACTAGAGCTGCTCCGCATCTCCCTGCTGCTCATCCA  
GTCTATGGCTGGAGCCCGTGCAGCTCCTCAGGAGCGTCTTCGCCAACAGCCTGGTGTACGGCGCCTCTGACAGCAAC  
GTCTATGACCTCCTAAAGGACCTAGAGGAAGGCATCCAAACGCTGATGGGGTGGAAGATGGCAGCCCCCGGACTGG  
GCAGATCTTCAAGCAGACCTACAGCAAGTTCGACACAACTACACAACGATGACGCACTACTCAAGAACTACGGG  
CTGCTCTACTGCTTCAGGAAAGACATGGACAAGGACAAGCATTCCTGCGCATCGAGCAATGCCGCTCTGTGGAGG  
GCAGGTGTGGCTTCTAGCTGCCCCGGGTGGCATCCCTGTGACCCCTCCCCAGTGCCTGTCTGGGCCTGGAAGTTGC  
CACTCCAGTGCCACCAACCTTGGACCAATAAATTAAACGGCACATTC

The predicted protein of FCTR4 (SEQ ID NO:8), having 246 amino acid residues, is shown in TABLE 4B.

**TABLE 4B**  
**FCTR4 Protein Sequence (SEQ ID NO:8)**

MATGSRTSLLLAFGLLCLPWLQEGSAFPTIPLSRLFDNAMLRAHRLHQLAYDTYQEFEEAYIPKEQKYSFLQNPQT  
SLCFSESIPTPSNREKTQQKSNELELLRISLLLIQSWLEPVQLLRVSFANSLVYGASDSNVYDLLKDLEEGIQTLMG  
WKMAAPGLGRSSSRPTASSTQHTTMTHTYSRTTGCTASGKTWTRTRHSCASSNAALWRAGVASSCPGGIPVTPPQ  
CLSWAWKLPLQCPPTLDQ

Growth hormone (GH) is synthesized by acidophilic or somatotrophic cells of the anterior pituitary gland. Human growth hormone has a molecular mass of 22,005 and contains 191 amino acid residues with 2 disulfide bridges. See, *e.g.*, Niall *et al.*, 1971 *Nat New Biol.* 230: 90-91 and Niall *et al.* 1971 *Proc Natl Acad Sci USA* 68: 866-870. Growth hormone pathogenesis is also known to be involved in various forms of growth misregulation, such as dwarfism and acromegaly. See, *e.g.*, OMIM Acc. No. 139250.

In a search of Sequence databases, it was found, for example as shown in TABLE 4C, that the nucleic acid sequence has 773 of 807 bases (95%) identical, and 773 of 807 bases (95%) positive, aligned in an identity search, to a sequence of a synthetic construct containing human growth factor (SEQ ID NO:30) (Genbank Acc. E00952). As indicated by the "Expect"

value, the probability of this alignment occurring by chance alone is  $3.9 \times 10^{-161}$ , an extremely low probability.

TABLE 4C: BlastN

(Query = FCTR4; Sbjct = Human Growth Factor Construct (SEQ ID NO:30))

gb:GENBANK-ID:E00952|acc:E00952 Plasmid DNA for amplifying human growth factor gene in E.coli - synthetic construct, 3477 bp.

Plus Strand HSPs:

Score = 3689 (553.5 bits), Expect =  $3.9 \times 10^{-161}$ , P =  $3.9 \times 10^{-161}$

Identities = 773/807 (95%), Positives = 773/807 (95%), Strand = Plus / Plus

```

Query:      1  GCCCAACTCCCCGAACCACTCAGGGTCCTGTGGACAGCTCACCTAGCGGCAATGGCTACA 60
             |||
Sbjct:    304  GCCCAACTCCCCGAACCACTCAGGGTCCTGTGGAC-GCTCACCTAGCTGCAATGGCTACA 362
             |||

Query:      61  GGCTCCCGGACGTCCCTGCTCCTGGCTTTTGGCTGCTCTGCCTGCCCTGGCTTCAAGAG 120
             |||
Sbjct:    363  GGCTCCCGGACGTCCCTGCTCCTGGCTTTTGGCTGCTCTGCCTGCCCTGGCTTCAAGAG 422
             |||

Query:     121  GGCAGTGCCTTCCCAACCAATTCCCTTATCCAGGCTTTTGGACAACGCTATGCTCCGCGCC 180
             |||
Sbjct:    423  GGCAGTGCCTTCCCAACCAATTCCCTTATCCAGGCTTTTGGACAACGCTAGTCTCCGCGCC 482
             |||

Query:     181  CATCGCTGCACCAGCTGGCATATGACACCTACCAGGAGTTTGAAGAAGCCTATATCCCA 240
             |||
Sbjct:    483  CATCGTCTGCACCAGCTGGCCTTTGACACCTACCAGGAGTTTGAAGAAGCCTATATCCCA 542
             |||

Query:     241  AAGGAACAGAAGTATTCATTCCCTGCAGAACCCCGAGACCTCCCTCTGCTTCTCAGAGTCT 300
             |||
Sbjct:    543  AAGGAACAGAAGTATTCATTCCCTGCAGAACCCCGAGACCTCCCTCTGTTTCTCAGAGTCT 602
             |||

Query:     301  ATTCCAACACCCTCCAACAGGGAGAAAACACAACAGAAATCCAACCTAGAGCTGCTCCGC 360
             |||
Sbjct:    603  ATTCCGACACCCTCCAACAGGGAGGAAACACAACAGAAATCCAACCTAGAGCTGCTCCGC 662
             |||

Query:     361  ATCTCCCTGCTGCTCATCCAGTCATGGCTGGAGCCCGTGCAGCTCCTCAGGAGCGTCTTC 420
             |||
Sbjct:    663  ATCTCCCTGCTGCTCATCCAGTCGTTGGCTGGAGCCCGTGCAGTTCTCAGGAGTGTCTTC 722
             |||

Query:     421  GCCAACAGCCTGGTGACGGCGCCTCTGACAGCAACGTCTATGACCTCCTAAAGGACCTA 480
             |||
Sbjct:    723  GCCAACAGCCTGGTGACGGCGCCTCTGACAGCAACGTCTATGACCTCCTAAAGGACCTA 782
             |||

Query:     481  GAGGAAGGCATCCAAACGCTGATGGGG----TGAAGATGGCAGCCCCGGACTGGGCAG 536
             |||
Sbjct:    783  GAGGAAGGCATCCAAACGCTGATGGGGAGGCTGGAAGATGGCAGCCCCGGACTGGGCAG 842
             |||

Query:     537  ATCTTCAAGCAGACCTACAGCAAGTTGACACAACTCACACAACGATGACGCACTACTC 596
             |||
Sbjct:    843  ATCTTCAAGCAGACCTACAGCAAGTTGACACAACTCACACAACGATGACGCACTACTC 902
             |||

Query:     597  AAGAACTACGGGCTGCTCTACTGCTTCAGGAAAGACATGGACAAGGACAAGACATTCTG 656
             |||
Sbjct:    903  AAGAACTACGGGCTGCTCTACTGCTTCAGGAAAGACATGGACAAGGTCGAGACATTCTG 962
             |||

Query:     657  CGCATCGAGCAATGCCGCTCTGTGGAGGGCAGGTGTGGCTTCTAGCTGCCCGGGTGGCAT 716
             |||
Sbjct:    963  CGCATCGTGCAGTGCCGCTCTGTGGAGGGCAGCTGTGGCTTCTAGCTGCCCGGGTGGCAT 1022
             |||

```

```

Query:   717 CCCTGTGACCCCTCCCCAGTGCCTGTCTCTGGGCCTGGAAGTTGCCACTCCAGTGCCCACC 776
          |||
Sbjct:  1023 CCCTGTGACCCCTCCCCAGTGCCTGTCTCTGGGCCTGGAAGTTGCCACTCCAGTGCCCACC 1082

Query:   777 AACCTGGACCAATAAA-TTAAACGGCA 803
          |||
Sbjct:  1083 AGCCTTGTCTAATAAAATTAAGTTGCA 1110

```

The full amino acid sequence of the Somotropin Homolog FCTR4 was found to have 220 of 244 amino acid residues (90%) identical, and 227 of 224 residues (93%) positive to the 245 amino acid residue human Placental Growth Hormone Isoform HGH-V3 Precursor (SEQ ID NO:31) (ptnr:SPTREMBL-ACC: AAB71829) (TABLE 4D). As indicated by the "Expect" value, the probability of this alignment occurring by chance alone is 4.2e-115, an extremely low probability.

**TABLE 4D: BlastX**

**(Query = FCTR4; Sbjct = Human HGH-V3 Precursor (SEQ ID NO:31))**

ptnr:SPTREMBL-ACC:014644 PLACENTAL GROWTH HORMONE ISOFORM HGH-V3 PRECURSOR -  
HOMO SAPIENS (HUMAN), 245 aa.

Plus Strand HSPs:

Score = 1142 (402.0 bits), Expect = 4.2e-115, P = 4.2e-115

Identities = 220/244 (90%), Positives = 227/244 (93%), Frame = +1

```

Query:   52 MATGSRTSLLLAFLGLCLPWLQEGSAFPTIPLSRFLDNAMLRAHRLHQLAYDTYQEFEEA 231
          |||
Sbjct:   1  MAAGSRTSLLLAFLGLCLSWLQEGSAFPTIPLSRFLDNAMLRARRLYQLAYDTYQEFEEA 60

Query:  232 YIPKEQKYSFLQNPQTSLCFSESIPTPSNREKTQQKSNLELLRISLLLIQSWLEPVQLLR 411
          |||
Sbjct:   61 YILKEQKYSFLQNPQTSLCFSESIPTPSNRVKTQQKSNLELLRISLLLIQSWLEPVQLLR 120

Query:  412 SVFANSLVYGASDSNVYDLLKDLEEGIQTLMGWKMAAPGLGRSSSRPTASSTQHTTMTMTH 591
          |||
Sbjct:  121 SVFANSLVYGASDSNVYRHLKDLEEGIQTLIGWKMAAPGLGRSSISPTASLTQNRTTMTMTH 180

Query:  592 YSRTTGCSTASGKTWTRTRHSCASSNAALWRAGVASSCPGGIPVTPPQCLSWAWKLPLQC 771
          |||
Sbjct:  181 CSRTTGCSTASGRTWTRSRHSCASCAALWRAAVASSCPGGIPVTPPQCLSWSWKVLQLQC 240

Query:  772 PPTL 783
          |||
Sbjct:  241 PPAL 244

```

TABLE 4E shows a multiple sequence alignment, with the FCTR4 protein of the invention shown in line 2, in a ClustalW analysis. GHV3\_HUMAN indicates the human Placental Growth Hormone Isoform HGH-V3 Precursor (SEQ ID NO:31) (ptnr:SPTREMBL-ACC: AAB71829).

TABLE 4E: ClustalW Analysis

GHV3_HUMAN	MAAGSRTSLLLAFGLLCLSWLQEGSAFPT I PL SRLFDNAMLRA <del>RL</del> YQLAYDTYQEFEEA
SOMA_Novel2	MATGSRTSLLLAFGLLCLPWLQEGSAFPT I PL SRLFDNAMLRA <del>RL</del> YQLAYDTYQEFEEA
GHV3_HUMAN	<del>Y</del> ILKEQKYSFLQNPQTSLCFSESIPTPSNRV <del>K</del> TQQKSNLELLRISLLL IQSWLEPVQLLR
SOMA_Novel2	<del>Y</del> IPKEQKYSFLQNPQTSLCFSESIPTPSNR <del>E</del> KTQQKSNLELLRISLLL IQSWLEPVQLLR
GHV3_HUMAN	SVFANSLVYGASDSNVYRHLKDLEEGIGTL <del>I</del> GWKMAAPGLGRSSISPTASLTQNRITMTH
SOMA_Novel2	SVFANSLVYGASDSNVYDL <del>L</del> KDLEEGIGTL <del>I</del> MGWKMAAPGLGRSSSRPTASSTQTHITMTH
GHV3_HUMAN	CSRTTGCSTASGR <del>T</del> WTRSRHSCASC <del>S</del> AALWRA <del>V</del> ASSCPGGIPVTPPQCLSW <del>S</del> W <del>K</del> VLLQC
SOMA_Novel2	Y <del>S</del> RTTGCSTASGR <del>T</del> WTRSRHSCASS <del>N</del> AALWRA <del>G</del> VASSCPGGIPVTPPQCLSW <del>A</del> W <del>K</del> EP <del>L</del> QC
GHV3_HUMAN	PPA <del>L</del> S-
SOMA_Novel2	PP <del>T</del> L <del>D</del> Q

Black outlined amino acids indicate potential regions of conserved sequence (ie structural or functional regions); Greyed amino acids can be mutated to a limited extent without altering protein structure or function (*i.e.* L to V, I, or M); non-highlighted amino acids can potentially be mutated to a much broader extent without altering structure or function.

Human GH binds 2 GHR (See, *e.g.*, OMIM Acc. 600946) molecules and induces signal transduction through receptor dimerization. At high concentrations, GH acts as an antagonist because of a large difference in affinities at the respective binding sites. See, Sundstrom *et al.* 1996 *J Biol Chem* 271: 32197-32203. This antagonist action can be enhanced further by reducing binding in the low-affinity binding site. A possible mechanism by which mutant, biologically inactive GH may have its effect is to act as an antagonist to the binding of normal GH to its receptor, GHR. See, *e.g.*, Sundstrom *et al.* (1996) A GH antagonist mutant, GLY120 to ARG, was crystallized with its receptor as a 1-to-1 complex and determined the crystal structure at 2.9-angstrom resolution. See, Sundstrom *et al.* 1996. The 1-to-1 complex with the agonist was remarkably similar to the native GHR 1-to-2 complex. A comparison between the two structures revealed only minimal differences in the conformations of the hormone or its receptor in the two complexes. The regulation of GH synthesis and release is modulated by a family of genes that include the transcription factors PROP1 (See, *e.g.*, OMIM Acc. OMIM 601538) and PIT1 (OMIM 173110). PROP1 and PIT1 regulate differentiation of pituitary cells into somatotrophs which synthesize and release GH. Genes that are important in the release of GH include the GHRH (OMIM 139190) and GHRHR (OMIM 139191) genes. After GHRH is synthesized and released from the hypothalamus, it travels to the anterior pituitary where it binds to GHRHR, resulting in transduction of a signal into the somatotroph which promotes release of presynthesized GH that is stored in secretory granules. Other gene products that are important in GH synthesis and

release are GHR and the growth hormone binding proteins (GHBP). The GHBP are derived from the membrane bound receptor (GHR) and they remain bound to GH in the circulation. Following binding of GH to two GHR molecules, the signal to produce IGF1 (OMIM 147440) is transduced. The GH molecules that are bound to membrane-anchored GH receptors can be released into the circulation by excision of the extracellular portion of the GHR molecules. At this point, the extracellular portion of the GHR, which is referred to as the GHBP, serves to stabilize GH in the circulation. The final genes in the GH synthetic pathway include IGF1 and its receptor (IGF1R; OMIM 147370) whose products stimulate growth in various tissues including bones and muscle. See, *e.g.*, Phillips, 1995 ; and Rimoin and Phillips, "Genetic disorders of the pituitary gland." In: Rimoin *et al.* (eds.) : Principles and Practice of Medical Genetics. Vol. I. New York: Churchill Livingstone (3rd ed.) 1997. Pp. 1331-1364.

FCTR3 and FCTR4 are useful in the treatment of growth hormone deficiency in dwarfism, retardation, etc. Additionally, they are useful in treating Acromegaly and other disorders involving tissue growth.

#### **FCTR5: Neutrophil Activating Peptide Homolog**

Included in the invention is FCTR5 (also referred to as AC013573\_A), a nucleic acid encoding a homolog of Neutrophil-Activating Peptide 2 (NAP-2). Antibodies that bind specifically to FCTR5 polypeptides, and fragments thereof, are included in the invention. The invention further includes fragments, homologs, analogs, and derivatives of FCTR5 nucleic acids, polypeptides, and antibodies.

NAP-2 is also known as platelet basic protein precursor (PBP), connective-tissue activating peptide III (CTAP-III), low-affinity platelet factor IV (LA-PF4), beta-thromboglobulin (beta-TG), fractalkine, dendrokinin, and thymus and activation regulated cytokine (TARC). The 465 residues of the FCTR5 nucleic acid (SEQ ID NO:9) are shown in TABLE 5A..

**TABLE 5A**  
**Human FCTR5 Nucleotide Sequence (SEQ ID NO:9)**

GGCAGCAACTCACCCCTCAAGAGGAAGTCTTCAGGCTCTAGAAACATCTTTANCTCGGGTTTCTGCACCATAAGCCT  
CAGACTCAATGCCACCCTGCAGCTGTGCCAGATCACTATGTGCCCTGCAGGTGCTGCTGTTGACTGTTCTGGGTTT  
CTCCACCAATGGACAACTAAGAGAAACATAGGGAAAAGGAAAGCTGTCCGTGTAGACAGTGACTTGTACACTGAA  
CTGCGTGTGCTGTATGTGAAGTCAACCTTTGTACTTGATCCAGAAACATCCACAATTTGGAGTTGGTCTCAGCAG  
GACCCCATTCAGCAAAGACGAAGTAATGTCTCTTGAGAGCCACTCTGTAAGATGGGAGAAAAAATCTGCCTGGA  
CCCAGATGCTCCAGAATCAATAAAATTGTACAGAAAATGTTGAAAGTTGATGAATTCATCTGGTTAATTTGTTAA  
CTTTCTGCT

The predicted translation product of FCTR5 is a polypeptide of 123 amino acids (SEQ ID NO:10), shown in TABLE 5B.

**TABLE 5B**  
**Human FCTR5 Protein Sequence (SEQ ID NO:10)**

MPPCSCARSLCALQVLLLTVLGSSSTNGQTKRNIGKRKAVRVDSDLYTELRCVYVKSTFVLDPNRNIHNLELVSAGP  
HCSKDEVMSLAELCKMGEKICLDPDAPRINKIVQKMLKVDEFIWLIC

FCTR5 is related to several different genes known to be associated with human diseases or disorders. Interleukin-8 (OMIM 146930), growth regulated gene (OMIM 155730); small inducible cytokine subfamily B (member 5, SCYB5) (OMIM 600324) and neutrophil activating protein-2 (OMIM 121010; also known as connective tissue-activating peptide III; CTAP3) are members of a large family of small secreted proteins (8-10 kD) with proinflammatory and reparative activities, including chemotaxis of neutrophils. Interleukin-8, otherwise known as neutrophil-activating peptide-1, is a tissue-derived peptide secreted by several types of cells in response to inflammatory stimuli. Therapeutic uses include (1) Hematopoiesis, immunotherapy, immunodeficiency diseases, all inflammatory diseases; (2) Cancer therapy; (3) Autoimmune diseases; and (4) Obesity, anorexia and body mass related disorders.

In a search of sequence databases, it was found, for example, that the FCTR5 nucleic acid sequence has homology to various fragments of the 688 basepair human Neutrophil-Stimulating Peptide 2 synthetic construct (SEQ ID NO:32) (Genbank-ID:A01319|acc:A01319). The first alignment in Table5C indicates that FCTR5 has 159 of 202 bases identical (78%), and 159 of 202 bases positive, when aligned in an identity search residues 149 to 350 of SEQ ID NO:32. As indicated by the "Expect" value, the probability of this alignment occurring by chance alone is  $1.0e-54$ , an extremely low probability. The second alignment in Table5C, with an expect value of  $1.0e-54$ , indicates that FCTR5 has 104 of 123 bases both identical and positive (84%), when aligned with residues 349 to 469 of SEQ ID NO:32. The third alignment in Table5C, with an expect value of  $1.0e-54$ , indicates that FCTR5 has 108 of 143 bases both identical and positive (75%), when aligned with residues 3 to 145 of SEQ ID NO:32. The BLASTN results of these two sequences are shown in TABLE 5C.

**TABLE 5C: BlastN**

**Query = FCTR5; Sbjct = Human Neutrophil-Stimulating Peptide 2 Construct (SEQ ID NO:32)**

gb:GENBANK-ID:A01319|acc:A01319 H.sapiens mRNA for recombinant neutrophil-stimulating peptide 2 - synthetic construct, 688 bp (RNA)  
Plus Strand HSPs:

Score = 623 (93.5 bits), Expect =  $1.0e-54$ , Sum P(3) =  $1.0e-54$

Identities = 159/202 (78%), Positives = 159/202 (78%), Strand = Plus / Plus

```

Query:   131 TGCTGTTGACTGTTCTGGGTTCCCTCCACCAATGGACAACTAAGAGAAACATAGGGAAAA 190
          ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct:   149 TGCTGCTGACTGCTCTGGCTTCCTCCACCAAAGGACAACTAAGAGAACTTGGCGAAAG 208

Query:   191 GGAAAGCTGTCCGTGTAGACAGTGACTTGTACACTGAACTGCGCTGCGTGTATGTGAAGT 250
          ||||| | || ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct:   209 GCAAAGAGGAAAGTCTAGACAGTGACTTGTATGCTGAACTCCGCTGCATGTGTATAAAGA 268

Query:   251 CAACCTTTGTACTTGATCCCAGAAACATCCACAATTGGAGTTGGTCTCAGCAGGACCCC 310
          ||||| || || ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct:   269 CAACCTCTGGAATTCATCCCAAAACATCCAAAGTTTGGAAGTGATCGGGAAAGGAACCC 328

Query:   311 ATTGCAGCAAAGACGAAGTAAT 332
          ||||| || || ||||| ||
Sbjct:   329 ATTGCAACCAAGTCGAAGTGAT 350

```

Score = 432 (64.8 bits), Expect = 1.0e-54, Sum P(3) = 1.0e-54

Identities = 104/123 (84%), Positives = 104/123 (84%), Strand = Plus / Plus

```

Query:   342 AGAGCCACTCTGTAAGATGGGAGAAAAAATCTGCCTGGACCCAGATGCTCCAGAATCAA 401
          | ||||| ||| | ||||| |||| | ||||| ||||| ||||| ||||| |||||
Sbjct:   349 ATAGCCCACTGAAGGATGGGAGGAAAA-TCTGCCTGGACCCAGATGCTCCAGAATCAA 407

Query:   402 TAAAATTGTACAGAAAATGTTGAAAGTTGATGAATTCATCTGGTTAATTTGTTAACTTTC 461
          ||||| ||||| ||| || ||||| || || ||||| || || ||||| |||||
Sbjct:   408 GAAAATTGTACAGAAAAAATTGGCAGGTGATGAAT-CTGCTGATTAAATTTGTTCTGTTTC 466

Query:   462 TGC 464
          |||
Sbjct:   467 TGC 469

```

Score = 362 (54.3 bits), Expect = 1.0e-54, Sum P(3) = 1.0e-54

Identities = 108/143 (75%), Positives = 108/143 (75%), Strand = Plus / Plus

```

Query:     5 GCAACTCACCTCAAGAGGAAGTCTTCAGGCTCTAGAAACATCTTTANCTCGGGTTTCTG 64
          ||||| ||||| || ||||| || || ||||| || || ||||| |||||
Sbjct:     3 GCAACTCACCTCACTCAGAGGTCTTCTGGTTCTGGAAACAACCTCTAGCTCAGCCTTCTC 62

Query:    65 CACCATAAGCCTCAGACTCAATGCCACCC-TGCA-G---CTGTGCCAGATCACTATGTGC 119
          ||||| ||||| ||||| || ||||| || || ||||| ||||| |||||
Sbjct:    63 CACCATGAGCCTCAGACTTGATACCACCCCTTCTGTAAACAGTGCGAGACCACTTCATGC 122

Query:   120 CCTGCAGGTGCTGCTGTGACTGT 143
          | ||||| ||||| || |||||
Sbjct:   123 CTTGCAGGTGCTGCTGCTT-CTGT 145

```

The full amino acid sequence of the FCTR5 polypeptide was found to have 68 of 120 amino acid residues (56%) identical, and 84 of 120 residues (70%) positive to the 128 amino acid residue human Neutrophil-Activating Peptide 2 (SEQ ID NO:33) (NAP-2) (ptnr:SWISSPROT-ACC:P02775). As indicated by the "Expect" value, the probability of this alignment occurring by chance alone is 4.8e-27, an extremely low probability. The BLASTN results of these two sequences are shown in TABLE 5D.

**TABLE 5D: BlastX**

**Query = FCTR5; Sbjct = Human Neutrophil-Activating Peptide 2 (SEQ ID NO:33)**

ptnr:SWISSPROT-ACC:P02775 PLATELET BASIC PROTEIN PRECURSOR (PBP) [CONTAINS:  
CONNECTIVE-TISSUE ACTIVATING PEPTIDE III (CTAP-III); LOW-AFFINITY PLATELET  
FACTOR IV (LA-PF4); BETA-THROMBOGLOBULIN (BETA-TG); NEUTROPHIL-ACTIVATING  
PEPTIDE 2 (NAP-2)] - Homo sapiens (Human), 128 aa.

Plus Strand HSPs:

Score = 311 (109.5 bits), Expect = 4.8e-27, P = 4.8e-27

Identities = 68/120 (56%), Positives = 84/120 (70%), Frame = +1

Query: 79 DSMPPCSCARSLCALQVLL-----TVLGSSTNGQTKRNIGKRKAVRVDSDLYTELCRVY 243  
 | + | | + | | | | | | | | | | | | | | | | | | + | | + | | | | | | +  
 Sbjct: 6 DTPSCNSARPLHALQVLLLLLLSLLLTALASSTKGQTKRNLAKGKEESLSDSLYAEALRCMC 65

Query: 244 VKSTFVLDPNRNIHNLELVSAGPHCSKDEVMSLAEPLCKMGKICLDPDAPRINKIVQKML 423  
 +|+| +|+| +|++ | |++ |++ | | | | | | | | | | | | | | |  
 Sbjct: 66 IKTTSGIHPKNIQSLVIGKGTNCQVEVIATL---KDGRKICLDPDAPRIKKIVQKKL 121

```

Query:      424  KVDE  435
            ||
Sbjct:      122  AGDE  125

```

A multiple sequence alignment is given in TABLE 5E, with the protein disclosed in SEQ ID NO:10 shown on line 1, in a ClustalW analysis comparing the protein of the invention with related protein sequences. PF4L\_PIG (SEQ ID NO:34) includes amino acid sequences from porcine platelet basic protein precursor (Genbank Acc. P43030, PID - g1172445). PF4L\_HUMAN (SEQ ID NO:33) includes amino acid sequences from Neutrophil-Activating Peptide 2 (Genbank Acc.: P02775, PID - g129874).

**TABLE 5E: ClustalW Analysis**

Novel\_NAP2      - - - - - Y P C S C A R S L C A L Q V L L L T V L G S S N G Q I K R N I G K R K A V F V - - - - - D S D L Y T E  
PF4L\_PIG      M S L R L G A I T S S C I T S S F P P V L Q V L L P L S L L T L T L V P A T M G A A K I E G - R - - - - - M A H V E  
PF4L\_HUMAN    M S L R L D T T P S C N S A R P L H A L Q V L L L L S L L T A L A S T I K G Q T K R N L A K G K E E S L D S D L Y A E

Novel\_NAP2      L R C V Y Y K S T F V L E D P R N I H N L E L V S A G P H C S K D E V M S L A E P L C K M I E K I C L D F D A P R I N K I  
PF4L\_PIG      L R C L C E N T V S G I T H P S N I Q S L E V I R A G A H C A K V E V I A T L K - - - - - N D K I C L D F D A P R I K K I  
PF4L\_HUMAN    L R C M C I K T T S G I H P K N I Q S L E V I G K G T H Q N Q V E V I A T L K - - - - - D C R K I C L D F D A P R I K K I

Novel\_NAP2      V Q K Y L K V D E F I W L I C  
PF4L\_PIG      V Q K I M E D G G S A A - - -  
PF4L\_HUMAN    V Q K K L A G D E S A D - - -

Black outlined amino acids indicate potential regions of conserved sequence (*i.e.* structural or functional regions); Greyed amino acids can be mutated to a limited extent without altering protein structure or function (*i.e.* L to V, I, or M); non-highlighted amino acids can potentially be mutated to a much broader extent without altering structure or function.



**FCTR6: Neutrophil Activating Peptide-2 Related Polypeptide**

Included in the invention is FCTR6, a second homolog of Neutrophil-Activating Peptide 2 (NAP-2). Antibodies that bind specifically to FCTR6 polypeptides, and fragments thereof, are included in the invention. The invention further includes fragments, homologs, analogs, and derivatives of FCTR6 nucleic acids, polypeptides, and antibodies.

NAP-2 is also known as platelet basic protein precursor (PBP), connective-tissue activating peptide III (CTAP-III), low-affinity platelet factor IV (LA-PF4), beta-thromboglobulin (Beta-TG), fractalkine, dendrokin, and thymus and activation regulated cytokine (TARC).

In one embodiment, FCTR6 nucleic acid (SEQ ID NO:11) is 463 nucleotides in length and encodes a neutrophil activating peptide homolog. Putative untranslated regions upstream from the start codon and downstream from the termination codon are underlined. Putative start and termination codons are shown in bold.

**TABLE 6A**  
**FCTR6 Nucleotide Sequence (SEQ ID NO:11)**

CTCAGTCTTCTCCACCATGAGCCTCAGACTCAAAGCCACCCCTTCTGTAACAGTGCCAGACCATTTCATGCCCTG  
CAGGTGTTGCTGCTTCTGTCACTGCTGCTGACCGCTCTGCCTTCTGTCACCAATGGGCAAAGCAAGAGAACTTAG  
GGAAAAGCAAAGCTGAATGTGTAGACAGTGACTTGTACATTGAACTGTGCTGCGTGTGTGTGAAGTCAGCCTCTGG  
AATTCATCCCAGCACTCATCATTTGGAAGTGGTCAGGTCAGGAGCCCTTGCAACAAAGTCCAAGTGATAGCCATG  
CTGAAAGATGGGAGGAAAATGTACCTGGACCCAGAAGTCCCAGAATCAAGAAGATAGTCCAGAAAATGTTGGAAG  
GTGATGGGTGAGGTGCTTAATCTGTTCACTTTCTGCCAAACCTGTTTACCTCCCAGGAAGGGCAGGATTTTAAAGC  
CTTGACT

The encoded FCTR6 protein (SEQ ID NO:12) having 127 amino acid residues is shown in TABLE 6B.

**TABLE 6B**  
**FCTR6 Protein Sequence (SEQ ID NO:12)**

MSLRKATPSCNSARPFFHALQVLLLLSLLLTALPSC TNGQSKRN LGKSKAECVDS DLYIELCCVCVKSASGIHPS  
THHLEVVRSGAPCNKVQVIAM LKDG RMYLDPEAPRIKKIVQKMLEGDGSGA

FCTR5 is related to several different genes known to be associated with human diseases or disorders. Interleukin-8 (OMIM 146930), growth regulated gene (OMIM 155730); small inducible cytokine subfamily B (member 5, SCYB5) (OMIM 600324) and neutrophil activating protein-2 (OMIM 121010; also known as connective tissue-activating peptide III; CTAP3) are members of a large family of small secreted proteins (8-10 kD) with proinflammatory and reparative activities, including chemotaxis of neutrophils. Interleukin-8, otherwise known as neutrophil-activating peptide-1, is a tissue-derived peptide secreted by several types of cells in response to inflammatory stimuli. Therapeutic uses include (1)

Hematopoiesis, immunotherapy, immunodeficiency diseases, all inflammatory diseases; (2) Cancer therapy; (3) Autoimmune diseases; and (4) Obesity, anorexia and body mass related disorders.

In a search of sequence databases, it was found, for example, that the nucleic acid sequence has 388 of 466 bases identical (83%), and 388 of 466 bases positive, when aligned in an identity search, to a 688 base pair human Neutrophil-Stimulating Peptide 2 synthetic construct (SEQ ID NO:32) (Genbank-ID:A01319|acc:A01319). As indicated by the "Expect" value, the probability of this alignment occurring by chance alone is 1.6e-66, an extremely low probability. The BLASTN results are shown in TABLE 6C.

**TABLE 6C: BlastN**

**Query = FCTR6; Sbjct = Human Neutrophil-Stimulating Peptide 2 Construct (SEQ ID NO:32)**

gb:GENBANK-ID:A01319|acc:A01319 H.sapiens mRNA for recombinant neutrophil-stimulating peptide 2 - synthetic construct, 688 bp (RNA)

Plus Strand HSPs:

Score = 1610 (241.6 bits), Expect = 1.6e-66, P = 1.6e-66

Identities = 388/466 (83%), Positives = 388/466 (83%), Strand = Plus / Plus

```

Query:      1 CTCAGTCTTCTCCACCATGAGCCTCAGACTCAAAGCCACCCCTTCCTGTAACAGTGCCAG 60
             ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct:     51 CTCAGCCTTCTCCACCATGAGCCTCAGACTTGATACCACCCCTTCCTGTAACAGTGCGAG 110

Query:     61 ACCATTTTCATGCCCTGCAGGTGTTGCTGCTTCTGTCACTGCTGCTGACCGCTCTGCCTTC 120
             ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct:    111 ACCACTTCATGCCTTGCAGGTGCTGCTGCTTCTGTCTATTGCTGCTGACTGCTCTGGCTTC 170

Query:    121 CTGCACCAATGGGCAAAGCAAGAGAACTTAGGGAAAAGCAAAGCTGAATGTGTAGACAG 180
             || ||||| || ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct:    171 CTCCACCAAAGGACAACTAAGAGAACTTGCGCAAAGGCAAAGAGGAAAGTCTAGACAG 230

Query:    181 TGAATTGTACATTGAACTGTGCTGCGTGTGTGTGAAGTCAGCCTCTGGAATTCATCCCAG 240
             ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct:    231 TGAATTGTATGCTGAACTCCGCTGCATGTGTATAAAGACAACCTCTGGAATTCATCCCAA 290

Query:    241 CA-C-TCATCA-TTTGGAAGTGGTCAGGTGAGGAGCCCTTGCAACAAAGTCCAAGTGAT 297
             || || || || ||||| || || || || ||||| ||||| ||||| ||||| |||||
Sbjct:    291 AAACATCCAAAGTTTGGAAGTGATCGGGAAAGGAACCCATTGCAACCAAGTCGAAGTGAT 350

Query:    298 AGCCATGCTGAAAGATGGGAGGAAAATGTACCTGGACCCAGAAGCTCCCAGAATCAAGAA 357
             ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct:    351 AGCCACACTGAAGGATGGGAGGAAAATCTGCCTGGACCCAGATGCTCCCAGAATCAAGAA 410

Query:    358 GATAGTCCAGAAAATGTTGGAAGGTGATGGGTGAGGTGCTTAATCTGTTCACCTTTCTGCC 417
             || || ||||| ||||| ||||| ||||| || || || ||||| ||||| |||||
Sbjct:    411 AATTGTACAGAAAAAATTGGCAGGTGATGAATCTGCTGATTAATTGTTCTGTTTCTGCC 470

Query:    418 AAACCTGTTTACCTCCCAGGAAGGGCAGGATTTTAAAGCCTTGACT 463
             ||||| || ||||| ||||| ||||| || ||||| || ||||| ||
Sbjct:    471 AAACCTCTTTAACTCCCAGGAAGGGTAGAATTTTGAACCTTGATT 516

```

**TABLE 6D: BlastX**

Sbjct: 121 LAGDES 126

26

TABLE 6E: ClustalW Analysis

NAP2_Novel	MSLRRLKATPSCNSARPFHALQVLLLSLLLTALPSCINGGSKRNLGKSKAECDSDLYE
PF4L_PIG	MSLRRLGAISSCTIISPPFVLOVLLPLSLLLTILVPATMGAAK-IEGR-----MAHVE
PF4L_HUMAN	MSLRRLDTTPSCNSARPLHALQVLLLSLLLTALASSTKGGIKRNLAKGKEESDSDLYAE
NAP2_Novel	LCCVCKSA SGIHPS-THHLEVYRSGAPCNKVQVIAMLDGRKMYLDPEAPRIKKIVQKM
PF4L_PIG	LRCLENTVSGIHPSNIQSLEVI RAGAHCAKVEVIATLKNDKKICLDPEAPRIKKIVQKI
PF4L_HUMAN	LRCVCKITTS GIIHPKNIQSLEVI GKGTHCNQVEVIATLKDGRKICLDPEAPRIKKIVQKK
NAP2_Novel	LEGDGSGA
PF4L_PIG	MEDGGSAA
PF4L_HUMAN	LAGDESAD

Black outlined amino acids indicate potential regions of conserved sequence (*i.e.* structural or functional regions); Greyed amino acids can be mutated to a limited extent without altering protein structure or function (*i.e.* L to V, I, or M); non-highlighted amino acids can potentially be mutated to a much broader extent without altering structure or function.

### FCTR X Nucleic Acids

TABLE 7 below summarizes the disclosed FCTR X nucleic acid sequences, encoded polypeptides, and the corresponding sequence identifier numbers (SEQ ID NOs) for the various disclosed sequences and clones containing these nucleic acids and polypeptides.

TABLE 7A: Summary Of Nucleic Acids And Proteins Of The Invention

FCTR X	Clone	DESCRIPTION of Homolog	NUCLEIC ACID SEQ ID NOs	POLYPEPTIDE SEQ ID NOs
FCTR1	AC009647-A	Neurite Outgrowth-Promoting Protein Homolog	1	2
FCTR2	AF202031-A	Sperm Antigen He-2	3	4
FCTR3	AC013601_A	Somatotropin Homologs	5	6
FCTR4	AC013601_B	S100 Cytokine Homologs	7	8
FCTR5	AC013573	Neutrophil Activating Peptide-2 Homolog	9	10
FCTR6	AL133124	Neutrophil Activating Peptide-2 Homolog	11	12
	Forward	Ag351 TaqMan oligo	13	
	Primer	Ag351 TaqMan oligo	14	
	Reverse	Ag351 TaqMan oligo	15	

**TABLE 7B: Summary of Query Sequences Disclosed**

Database	Acc. No.	Gene Name	Species	SEQ ID NO.
GENBANK	X55110	neurite outgrowth-promoting protein	Human	16
SWISSPROT	P21741	midkine precursor	Human	17
GENBANK	P12025	midkine precursor	Mouse	18
GENBANK	BAA83783	midkine precursor	Rat	19
GENBANK	P24052	midkine precursor	Chick	20
GENBANK	P48531	pleiotrophic factor alpha 2 precursor	Xenopus	21
GENBANK	X67697	HE2 mRNA	Human	22
GENBANK	Q08648	Sperma antigen HE2 precursor	Human	23
GENBANK	I37454	HE2 protein	Human	24
GENBANK	E00952	synthetic growth factor construct	Human	25
SWISSPROT	P01241	Somatotropin Precursor	Human	26
GENBANK	P33093	Somatotropin Precursor	Mouse	27
GENBANK	P01244	Somatotropin Precursor	Rat	28
GENBANK	P06880	Somatotropin Precursor	Mouse	29
GENBANK	E00952	Growth factor construct	Human	30
GENBANK	AAB71829	GROWTH HORMONE HGH-V3	Human	31
GENBANK	A01319	synthetic Neutrophil-Stimulating Peptide 2	Human	32
GENBANK	P02775	Neutrophil-Activating Peptide 2	Human	33
GENBANK	P43030	platelet basic protein precursor	Pig	34

**FCRX Nucleic Acids**

The novel nucleic acids of the invention include those that encode a FCRX polypeptide or biologically active portions thereof. The nucleic acids include nucleic acids encoding FCRX polypeptides that include the amino acid sequence of one or more of SEQ ID NOS:2, 4, 6, 8, 10 and 12. In some embodiments, a nucleic acid encoding a polypeptide having the amino acid sequence of one or more of SEQ ID NOS:2, 4, 6, 8, 10 and 12 includes the nucleic acid sequence of any of SEQ ID NOS:1, 3, 5, 7, 9 and 11, or a fragment thereof.

Additionally, a FCRX nucleic acid of the invention includes mutant or variant nucleic acids of any of SEQ ID NOS:1, 3, 5, 7, 9 and 11, or a fragment thereof, any of whose bases may be changed from the disclosed sequence while still encoding a protein that maintains its

FCTR<sub>X</sub> -like activities and physiological functions. The invention further includes the complement of the nucleic acid sequence of any of SEQ ID NOS:1, 3, 5, 7, 9 and 11, including fragments, derivatives, analogs and homolog thereof. The invention additionally includes nucleic acids or nucleic acid fragments, or complements thereto, whose structures include chemical modifications.

A FCTR<sub>X</sub> nucleic acid of the invention can encode a mature form of a FCTR<sub>X</sub> polypeptide. As used herein, a "mature" form of a polypeptide or protein is the product of a naturally occurring polypeptide or precursor form or proprotein. The naturally occurring polypeptide, precursor or proprotein includes, by way of nonlimiting example, the full length gene product, encoded by the corresponding gene. Alternatively, it may be defined as the polypeptide, precursor or proprotein encoded by an open reading frame described herein. The product "mature" form arises, again by way of nonlimiting example, as a result of one or more naturally occurring processing steps as they may take place within the cell, or host cell, in which the gene product arises. Examples of such processing steps leading to a "mature" form of a polypeptide or protein include the cleavage of the N-terminal methionine residue encoded by the initiation codon of an open reading frame, or the proteolytic cleavage of a signal peptide or leader sequence. Thus a mature form arising from a precursor polypeptide or protein that has residues 1 to N, where residue 1 is the N-terminal methionine, would have residues 2 through N remaining after removal of the N-terminal methionine. Alternatively, a mature form arising from a precursor polypeptide or protein having residues 1 to N, in which an N-terminal signal sequence from residue 1 to residue M is cleaved, would have the residues from residue M+1 to residue N remaining. Additionally, a "mature" protein or fragment may arise from a cleavage event other than removal of an initiating methionine or removal of a signal peptide. Further as used herein, a "mature" form of a polypeptide or protein may arise from a step of post-translational modification other than a proteolytic cleavage event. Such additional processes include, by way of non-limiting example, glycosylation, myristylation or phosphorylation. In general, a mature polypeptide or protein may result from the operation of only one of these processes, or a combination of any of them.

Also included are nucleic acid fragments sufficient for use as hybridization probes to identify nucleic acids encoding FCTR<sub>X</sub> polypeptides (*e.g.*, a FCTR<sub>X</sub> mRNA encoding SEQ ID NO:2 or SEQ ID NO:4) and fragments for use as polymerase chain reaction (PCR) primers for the amplification or mutation of FCTR<sub>X</sub> nucleic acid molecules. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (*e.g.*, cDNA or genomic DNA), RNA molecules (*e.g.*, mRNA), analogs of the DNA or RNA generated using

nucleotide analogs, and derivatives, fragments and homologs thereof. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

"Probes" refer to nucleic acid sequences of variable length, preferably between at least about 10 nucleotides (nt), 100 nt, or as many as about, *e.g.*, 6,000 nt, depending on use.

Probes are used in the detection of identical, similar, or complementary nucleic acid sequences. Longer length probes are usually obtained from a natural or recombinant source (although they may be prepared by chemical synthesis as well), are highly specific and much slower to hybridize than oligomers. Probes may be single- or double-stranded and designed to have specificity in PCR, membrane-based hybridization technologies, or ELISA-like technologies.

An "isolated" nucleic acid molecule is one that is separated from other nucleic acid molecules that are present in the natural source of the nucleic acid. Examples of isolated nucleic acid molecules include, but are not limited to, recombinant DNA molecules contained in a vector, recombinant DNA molecules maintained in a heterologous host cell, partially or substantially purified nucleic acid molecules, and synthetic DNA or RNA molecules. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated FCTRX nucleic acid molecule can contain less than about 50 kb, 25 kb, 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material or culture medium when produced by recombinant techniques, or of chemical precursors or other chemicals when chemically synthesized.

A nucleic acid molecule of the present invention, *e.g.*, a nucleic acid molecule having the nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9 and 11, or a complement of any of this nucleotide sequence, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or a portion of the nucleic acid sequence of any of SEQ ID NOS:1, 3, 5, 7, 9 and 11 as a hybridization probe, FCTRX nucleic acid sequences can be isolated using standard hybridization and cloning techniques (*e.g.*, as described in Sambrook *et al.*, eds., MOLECULAR CLONING: A LABORATORY MANUAL 2<sup>nd</sup> Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989; and Ausubel, *et al.*, eds., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993.)

A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to FCTR<sub>X</sub> nucleotide sequences can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA synthesizer.

As used herein, the term “oligonucleotide” refers to a series of linked nucleotide residues, which oligonucleotide has a sufficient number of nucleotide bases to be used in a PCR reaction. A short oligonucleotide sequence may be based on, or designed from, a genomic or cDNA sequence and is used to amplify, confirm, or reveal the presence of an identical, similar or complementary DNA or RNA in a particular cell or tissue. Oligonucleotides comprise portions of a nucleic acid sequence having about 10 nt, 50 nt, or 100 nt in length, preferably about 15 nt to 30 nt in length. In one embodiment, an oligonucleotide comprising a nucleic acid molecule less than 100 nt in length would further comprise at least 6 contiguous nucleotides of any of SEQ ID NOS:1, 3, 5, 7, 9 and 11, or a complement thereof. Oligonucleotides may be chemically synthesized and may be used as probes.

In another embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule that is a complement of the nucleotide sequence shown in any of SEQ ID NOS:1, 3, 5, 7, 9 and 11. In another embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule that is a complement of the nucleotide sequence shown in any of SEQ ID NOS:1, 3, 5, 7, 9 and 11, or a portion of this nucleotide sequence. A nucleic acid molecule that is complementary to the nucleotide sequence shown in is one that is sufficiently complementary to the nucleotide sequence shown in of any of SEQ ID NOS:1, 3, 5, 7, 9 and 11 that it can hydrogen bond with little or no mismatches to the nucleotide sequence shown in of any of SEQ ID NOS:1, 3, 5, 7, 9 and 11, thereby forming a stable duplex.

As used herein, the term “complementary” refers to Watson-Crick or Hoogsteen base pairing between nucleotides units of a nucleic acid molecule, and the term “binding” means the physical or chemical interaction between two polypeptides or compounds or associated polypeptides or compounds or combinations thereof. Binding includes ionic, non-ionic, van der Waals, hydrophobic interactions, etc. A physical interaction can be either direct or indirect. Indirect interactions may be through or due to the effects of another polypeptide or compound. Direct binding refers to interactions that do not take place through, or due to, the



effect of another polypeptide or compound, but instead are without other substantial chemical intermediates.

Moreover, the nucleic acid molecule of the invention can comprise only a portion of the nucleic acid sequence of any of SEQ ID NOS:1, 3, 5, 7, 9 and 11, *e.g.*, a fragment that can be used as a probe or primer, or a fragment encoding a biologically active portion of a FCTR<sub>X</sub> polypeptide. Fragments provided herein are defined as sequences of at least 6 (contiguous) nucleic acids or at least 4 (contiguous) amino acids, a length sufficient to allow for specific hybridization in the case of nucleic acids or for specific recognition of an epitope in the case of amino acids, respectively, and are at most some portion less than a full length sequence. Fragments may be derived from any contiguous portion of a nucleic acid or amino acid sequence of choice. Derivatives are nucleic acid sequences or amino acid sequences formed from the native compounds either directly or by modification or partial substitution. Analogs are nucleic acid sequences or amino acid sequences that have a structure similar to, but not identical to, the native compound but differs from it in respect to certain components or side chains. Analogs may be synthetic or from a different evolutionary origin and may have a similar or opposite metabolic activity compared to wild type.

Derivatives and analogs may be full length or other than full length, if the derivative or analog contains a modified nucleic acid or amino acid, as described below. Derivatives or analogs of the nucleic acids or proteins of the invention include, but are not limited to, molecules comprising regions that are substantially homologous to the nucleic acids or proteins of the invention, in various embodiments, by at least about 70%, 80%, 85%, 90%, 95%, 98%, or even 99% identity (with a preferred identity of 80-99%) over a nucleic acid or amino acid sequence of identical size or when compared to an aligned sequence in which the alignment is done by a computer homology program known in the art, or whose encoding nucleic acid is capable of hybridizing to the complement of a sequence encoding the aforementioned proteins under stringent, moderately stringent, or low stringent conditions. See *e.g.* Ausubel, *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993, and below. An exemplary program is the Gap program (Wisconsin Sequence Analysis Package, Version 8 for UNIX, Genetics Computer Group, University Research Park, Madison, WI) using the default settings, which uses the algorithm of Smith and Waterman (Adv. Appl. Math., 1981, 2: 482-489, which is incorporated herein by reference in its entirety).

A "homologous nucleic acid sequence" or "homologous amino acid sequence," or variations thereof, refer to sequences characterized by a homology at the nucleotide level or

amino acid level as discussed above. Homologous nucleotide sequences encode those sequences coding for isoforms of a FCTR<sub>X</sub> polypeptide. Isoforms can be expressed in different tissues of the same organism as a result of, for example, alternative splicing of RNA. Alternatively, isoforms can be encoded by different genes. In the present invention, homologous nucleotide sequences include nucleotide sequences encoding for a FCTR<sub>X</sub> polypeptide of species other than humans, including, but not limited to, mammals, and thus can include, *e.g.*, mouse, rat, rabbit, dog, cat, cow, horse, and other organisms. Homologous nucleotide sequences also include, but are not limited to, naturally occurring allelic variations and mutations of the nucleotide sequences set forth herein. A homologous nucleotide sequence does not, however, include the nucleotide sequence encoding human FCTR<sub>X</sub> protein. Homologous nucleic acid sequences include those nucleic acid sequences that encode conservative amino acid substitutions (see below) in any of SEQ ID NOS:2, 4, 6, 8, 10 and 12 as well as a polypeptide having FCTR<sub>X</sub> activity. Biological activities of the FCTR<sub>X</sub> proteins are described herein.

As used herein, "identical" residues correspond to those residues in a comparison between two sequences where the equivalent nucleotide base or amino acid residue in an alignment of two sequences is the same residue. Residues are alternatively described as "similar" or "positive" when the comparisons between two sequences in an alignment show that residues in an equivalent position in a comparison are either the same amino acid or a conserved amino acid as defined below.

The nucleotide sequence determined from the cloning of the human FCTR<sub>X</sub> gene allows for the generation of probes and primers designed for use in identifying the cell types disclosed and/or cloning FCTR<sub>X</sub> protein homologues in other cell types, *e.g.*, from other tissues, as well as FCTR<sub>X</sub> homologues from other mammals. The probe/primer typically comprises a substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12, 25, 50, 100, 150, 200, 250, 300, 350 or 400 or more consecutive sense strand nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9 and 11; or an anti-sense strand nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9 and 11; or of a naturally occurring mutant of SEQ ID NOS:1, 3, 5, 7, 9 and 11.

Probes based on a human FCTR<sub>X</sub> nucleotide sequence can be used to detect transcripts or genomic sequences encoding the same or homologous proteins. In various embodiments, the probe further comprises a label group attached thereto, *e.g.*, the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can

be used as a part of a diagnostic test kit for identifying cells or tissue which misexpress a FCTR<sub>X</sub> protein, such as by measuring a level of a FCTR<sub>X</sub> protein-encoding nucleic acid in a sample of cells from a subject *e.g.*, detecting mRNA levels or determining whether a genomic FCTR<sub>X</sub> gene has been mutated or deleted.

"A polypeptide having a biologically active portion of a FCTR<sub>X</sub>" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a polypeptide of the present invention, including mature forms, as measured in a particular biological assay, with or without dose dependency. A nucleic acid fragment encoding a "biologically active portion of a FCTR<sub>X</sub> polypeptide" can be prepared by isolating a portion of SEQ ID NOS:1 or 3 that encodes a polypeptide having a FCTR<sub>X</sub> polypeptide biological activity such as those disclosed herein, expressing the encoded portion of FCTR<sub>X</sub> protein (*e.g.*, by recombinant expression *in vitro*) and assessing the activity of the encoded portion of the FCTR<sub>X</sub> polypeptide.

### FCTR<sub>X</sub> Variants

The invention further encompasses nucleic acid molecules that differ from the disclosed FCTR<sub>X</sub> nucleotide sequences due to degeneracy of the genetic code. These nucleic acids thus encode the same FCTR<sub>X</sub> protein as that encoded by the nucleotide sequence shown in SEQ ID NOS:1, 3, 5, 7, 9 and 11. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence shown in any of SEQ ID NOS:2, 4, 6, 8, 10 and 12.

In addition to the human FCTR<sub>X</sub> nucleotide sequence shown in any of SEQ ID NOS:1, 3, 5, 7, 9 and 11, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of a FCTR<sub>X</sub> may exist within a population (*e.g.*, the human population). Such genetic polymorphism in the FCTR<sub>X</sub> gene may exist among individuals within a population due to natural allelic variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising an open reading frame encoding a FCTR<sub>X</sub> protein, preferably a mammalian FCTR<sub>X</sub> protein. Such natural allelic variations can typically result in 1-5% variance in the nucleotide sequence of the FCTR<sub>X</sub> gene. Any and all such nucleotide variations and resulting amino acid polymorphisms in the FCTR<sub>X</sub> gene that are the result of natural allelic variation and that do not alter the functional activity of the FCTR<sub>X</sub> polypeptide are intended to be within the scope of the invention.

Moreover, nucleic acid molecules encoding FCTR<sub>X</sub> proteins from other species, and thus that have a nucleotide sequence that differs from the human sequence of any of SEQ ID

NOS:1, 3, 5, 7, 9 and 11, are intended to be within the scope of the invention. Nucleic acid molecules corresponding to natural allelic variants and homologues of the FCTR<sub>X</sub> cDNAs of the invention can be isolated based on their homology to the human FCTR<sub>X</sub> nucleic acids disclosed herein using the human cDNAs, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions.

In another embodiment, an isolated nucleic acid molecule of the invention is at least 6 nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence of any of SEQ ID NOS:1, 3, 5, 7, 9 and 11. In another embodiment, the nucleic acid is at least 10, 25, 50, 100, 250, 500 or 750 nucleotides in length. In another embodiment, an isolated nucleic acid molecule of the invention hybridizes to the coding region. As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences that exceed a minimum degree of similarity to each other typically remain hybridized to each other. For example, depending on the degree of stringency imposed, nucleotide sequences at least about 60% similar to each other may hybridize.

As used herein, the phrase "stringent hybridization conditions" refers to conditions under which a probe, primer or oligonucleotide will hybridize to a target sequence; optimally the probe will hybridize to no other sequences, and more generally will not hybridize to sequences below a specified degree of similarity to the probe. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures than shorter sequences. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point ( $T_m$ ) for the specific sequence at a defined ionic strength and pH. The  $T_m$  is the temperature (under defined ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to the target sequence hybridize to the target sequence at equilibrium. Since the target sequences are generally present at excess, at  $T_m$ , 50% of the probes are occupied at equilibrium. Typically, stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes, primers or oligonucleotides (*e.g.*, 10 nt to 50 nt) and at least about 60°C for longer probes, primers and oligonucleotides. Stringent conditions may also be achieved with the addition of destabilizing agents, such as formamide.

Stringent conditions such as described above are known to those skilled in the art and can be found in CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, N.Y.

(1989), 6.3.1-6.3.6. Preferably, the conditions are such that sequences at least about 65%, 70%, 75%, 85%, 90%, 95%, 98%, or 99% identical to each other typically remain hybridized to each other. A non-limiting example of stringent hybridization conditions is hybridization in a high salt buffer comprising 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 mg/ml denatured salmon sperm DNA at 65°C. This hybridization is followed by one or more washes in 0.2X SSC, 0.01% BSA at 50°C. An isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of any of SEQ ID NOS:1, 3, 5, 7, 9 and 11 corresponds to a naturally occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (*e.g.*, encodes a natural protein).

Homologs (*i.e.*, nucleic acids encoding FCTRX proteins derived from species other than human) or other related sequences (*e.g.*, paralogs) can be obtained by low, moderate or high stringency hybridization with all or a portion of the particular human sequence as a probe using methods well known in the art for nucleic acid hybridization and cloning.

In a second embodiment, a nucleic acid sequence that is hybridizable to the nucleic acid molecule comprising the nucleotide sequence of any of SEQ ID NOS:1, 3, 5, 7, 9 and 11, or fragments, analogs or derivatives thereof, under conditions of moderate stringency is provided. A non-limiting example of moderate stringency hybridization conditions are hybridization in 6X SSC, 5X Denhardt's solution, 0.5% SDS and 100 mg/ml denatured salmon sperm DNA at 55°C, followed by one or more washes in 1X SSC, 0.1% SDS at 37°C. Other conditions of moderate stringency that may be used are well known in the art. See, *e.g.*, Ausubel *et al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY.

In a third embodiment, a nucleic acid that is hybridizable to the nucleic acid molecule comprising the nucleotide sequence of any of SEQ ID NOS:1, 3, 5, 7, 9 and 11, or fragments, analogs or derivatives thereof, under conditions of low stringency, is provided. A non-limiting example of low stringency hybridization conditions are hybridization in 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 mg/ml denatured salmon sperm DNA, 10% (wt/vol) dextran sulfate at 40°C, followed by one or more washes in 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS at 50°C. Other conditions of low stringency that may be used are well known in the art (*e.g.*, as employed for cross-species hybridizations). See, *e.g.*, Ausubel *et al.* (eds.), 1993, CURRENT

PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY; Shilo and Weinberg, 1981, *Proc Natl Acad Sci USA* 78: 6789-6792.

### Conservative Mutations

In addition to naturally-occurring allelic variants of a FCTR<sub>X</sub> nucleotide sequence, *e.g.*, a gene sequence, that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequence of any of SEQ ID NOS:1, 3, 5, 7, 9 and 11, thereby leading to changes in the amino acid sequence of the encoded FCTR<sub>X</sub> protein, without altering the functional ability of the FCTR<sub>X</sub> protein. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in the sequence of any of SEQ ID NOS:1, 3, 5, 7, 9 and 11. A "non-essential" amino acid residue is a residue at a position in the sequence that can be altered from the wild-type sequence of the FCTR<sub>X</sub> polypeptide without altering the biological activity, whereas an "essential" amino acid residue is a residue at a position that is required for biological activity. For example, amino acid residues that are conserved among members of a family of FCTR<sub>X</sub> proteins, of which the FCTR<sub>X</sub> proteins of the present invention are members, are predicted to be particularly unamenable to alteration.

For example, a FCTR<sub>X</sub> protein according to the present invention can contain at least one domain that is a typically conserved region in a FCTR<sub>X</sub> protein family member. As such, these conserved domains are not likely to be amenable to mutation. Other amino acid residues, however, (*e.g.*, those that are poorly conserved among members of the FCTR<sub>X</sub> protein family) may not be as essential for activity and thus are more likely to be amenable to alteration.

Another aspect of the invention pertains to nucleic acid molecules encoding FCTR<sub>X</sub> proteins that contain changes in amino acid residues relative to the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4 that are not essential for activity. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a protein, wherein the protein comprises an amino acid sequence at least about 75% similar to the amino acid sequence of any of SEQ ID NOS:2, 4, 6, 8, 10 and 12. Preferably, the protein encoded by the nucleic acid is at least about 80% identical to any of SEQ ID NOS:2, 4, 6, 8, 10 and 12, more preferably at least about 90%, 95%, 98%, and most preferably at least about 99% identical to SEQ ID NO:2.

An isolated nucleic acid molecule encoding a protein homologous to the protein of any of SEQ ID NOS:2, 4, 6, 8, 10 and 12 can be created by introducing one or more nucleotide

substitutions, additions or deletions into the corresponding nucleotide sequence, such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein.

Mutations can be introduced into SEQ ID NOS:1, 3, 5, 7, 9 and 11 by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. Certain amino acids have side chains with more than one classifiable characteristic. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, tryptophan, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tyrosine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in a FCTR<sub>X</sub> polypeptide is replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a FCTR<sub>X</sub> coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for FCTR<sub>X</sub> polypeptide biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NOS:1, 3, 5, 7, 9 and 11 the encoded protein can be expressed by any recombinant technology known in the art and the activity of the protein can be determined.

The relatedness of amino acid families may also be determined based on side chain interactions. Substituted amino acids may be fully conserved "strong" residues or fully conserved "weak" residues. The "strong" group of conserved amino acid residues may be any one of the following groups: STA, NEQK, NHQK, NDEQ, QHRK, MILV, MILF, HY, FYW, wherein the single letter amino acid codes are grouped by those amino acids that may be substituted for each other. Likewise, the "weak" group of conserved residues may be any one of the following: CSA, ATV, SAG, STNK, STPA, SGND, SNDEQK, NDEQHK, NEQHRK, VLIM, HFY.

In one embodiment, a mutant FCTR<sub>X</sub> polypeptide can be assayed for (1) the ability to form protein:protein interactions with other FCTR<sub>X</sub> proteins, other cell-surface proteins, or biologically active portions thereof, (2) complex formation between a mutant FCTR<sub>X</sub> protein

and a FCTR<sub>X</sub> receptor; (3) the ability of a mutant FCTR<sub>X</sub> protein to bind to an intracellular target protein or biologically active portion thereof; (*e.g.*, avidin proteins); (4) the ability to bind BRA protein; or (5) the ability to specifically bind an antibody to a FCTR<sub>X</sub> polypeptide.

In other embodiments, a mutant FCTR<sub>X</sub> protein can be assayed for its ability to induce tumor formation, or to transform cells, such as NIH 3T3 cells, as described in the Examples below.

### **Antisense FCTR<sub>X</sub> Nucleic Acids**

Another aspect of the invention pertains to isolated antisense nucleic acid molecules that are hybridizable to or complementary to a FCTR<sub>X</sub> nucleic acid, *e.g.*, the antisense nucleic acid can be complementary to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9 and 11, or fragments, analogs or derivatives thereof. An "antisense" nucleic acid includes a nucleotide sequence that is complementary to a "sense" nucleic acid encoding a protein, *e.g.*, complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. In specific aspects, antisense nucleic acid molecules are provided that comprise a sequence complementary to at least about 10, 25, 50, 100, 250 or 500 nucleotides or an entire FCTR<sub>X</sub> coding strand, or to only a portion thereof. Nucleic acid molecules encoding fragments, homologs, derivatives and analogs of a FCTR<sub>X</sub> protein of any of SEQ ID NOS:2, 4, 6, 8, 10 and 12 or antisense nucleic acids complementary to a FCTR<sub>X</sub> nucleic acid sequence of SEQ ID NOS:1, 3, 5, 7, 9 and 11 are additionally provided.

In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding a FCTR<sub>X</sub> polypeptide. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues (*e.g.*, the protein coding region of a FCTR<sub>X</sub> polypeptide that corresponds to any of SEQ ID NOS:2, 4, 6, 8, 10 and 12). In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding a FCTR<sub>X</sub> polypeptide. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (*i.e.*, also referred to as 5' and 3' untranslated regions).

The FCTR<sub>X</sub> coding strand sequences disclosed herein (*e.g.*, SEQ ID NOS:1, 3, 5, 7, 9 and 11) allow for antisense nucleic acids to be designed according to the rules of Watson and Crick or Hoogsteen base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of a FCTR<sub>X</sub> mRNA. Alternatively, the antisense nucleic acid molecule can be an oligonucleotide that is antisense to only a portion of the coding or



noncoding region of a FCTR<sub>X</sub> mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of the FCTR<sub>X</sub> mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length.

An antisense nucleic acid of the invention can be constructed using chemical synthesis or enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, *e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used.

Examples of modified nucleotides that can be used to generate the antisense nucleic acid include: 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a FCTR<sub>X</sub> protein to thereby inhibit expression of the protein, *e.g.*, by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule that binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense

nucleic acid molecules of the invention includes direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, *e.g.*, by linking the antisense nucleic acid molecules to peptides or antibodies that bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are generally preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an  $\alpha$ -anomeric nucleic acid molecule. An  $\alpha$ -anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual  $\beta$ -units, the strands run parallel to each other (Gaultier *et al.* (1987) *Nucleic Acids Res* 15: 6625-6641). The antisense nucleic acid molecule can also comprise a 2'-O-methylribonucleotide (Inoue *et al.* (1987) *Nucleic Acids Res* 15: 6131-6148) or a chimeric RNA-DNA analogue (Inoue *et al.* (1987) *FEBS Lett* 215: 327-330).

Such modifications include, by way of nonlimiting example, modified bases, and nucleic acids whose sugar phosphate backbones are modified or derivatized. These modifications are carried out at least in part to enhance the chemical stability of the modified nucleic acid, such that they may be used, for example, as antisense binding nucleic acids in therapeutic applications in a subject.

Also within the invention is a FCTR<sub>X</sub> ribozymes. Ribozymes are catalytic RNA molecules with ribonuclease activity that are capable of cleaving a single-stranded nucleic acid, such as a FCTR<sub>X</sub> mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes (described in Haselhoff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave the FCTR<sub>X</sub> mRNA transcripts to thereby inhibit translation of the FCTR<sub>X</sub> mRNA. A ribozyme having specificity for a FCTR<sub>X</sub>-encoding nucleic acid can be designed based upon the nucleotide sequence of a FCTR<sub>X</sub> nucleic acid disclosed herein (*i.e.*, SEQ ID NOS:1, 3, 5, 7, 9 and 11). For example, a derivative of a Tetrahymena L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a FCTR<sub>X</sub>-encoding mRNA. See, *e.g.*, Cech *et al.*, U.S. Pat. No. 4,987,071; and Cech *et al.* U.S. Pat. No. 5,116,742. Alternatively, a FCTR<sub>X</sub> mRNA can be used to select a catalytic RNA having a

specific ribonuclease activity from a pool of RNA molecules. See, *e.g.*, Bartel *et al.*, (1993) *Science* 261:1411-1418.

Alternatively, FCTR<sub>X</sub> gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of a FCTR<sub>X</sub> gene (*e.g.*, the FCTR<sub>X</sub> gene promoter and/or enhancers) to form triple helical structures that prevent transcription of the FCTR<sub>X</sub> gene in target cells. See generally, Helene. (1991) *Anticancer Drug Des.* 6: 569-84; Helene. *et al.* (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher (1992) *Bioassays* 14: 807-15.

In various embodiments, the FCTR<sub>X</sub> nucleic acids can be modified at the base moiety, sugar moiety or phosphate backbone to improve, *e.g.*, the stability, hybridization, or solubility of the molecule. For example, the deoxyribosephosphate backbone of the nucleic acids can be modified to generate peptide nucleic acids (see Hyrup *et al.* (1996) *Bioorg Med Chem* 4: 5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, *e.g.*, DNA mimics, in which the deoxyribosephosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup *et al.* (1996) above; Perry-O'Keefe *et al.* (1996) *Proc. Nat. Acad. Sci. (USA)* 93: 14670-675.

PNAs based on FCTR<sub>X</sub> nucleic acids can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, *e.g.*, inducing transcription or translation arrest or inhibiting replication. PNA based on FCTR<sub>X</sub> nucleic acids can also be used, *e.g.*, in the analysis of single base pair mutations in a gene by, *e.g.*, PNA directed PCR clamping; as artificial restriction enzymes when used in combination with other enzymes, *e.g.*, S1 nucleases (Hyrup B. (1996) above); or as probes or primers for DNA sequence and hybridization (Hyrup *et al.* (1996), above; Perry-O'Keefe (1996), above).

In a further embodiment, PNAs of FCTR<sub>X</sub> nucleic acids can be modified, *e.g.*, to enhance their stability or cellular uptake, by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras of the nucleic acids can be generated that may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, *e.g.*, RNase H and DNA polymerases, to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of

base stacking, number of bonds between the nucleobases, and orientation (Hyrup (1996) above). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup (1996) above and Finn *et al.* (1996) *Nucl Acids Res* 24: 3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry, and modified nucleoside analogs, *e.g.*, 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used between the PNA and the 5' end of DNA (Mag *et al.* (1989) *Nucl Acid Res* 17: 5973-88). PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn *et al.* (1996) above). Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment. See, Petersen *et al.* (1975) *Bioorg Med Chem Lett* 5: 1119-11124.

In other embodiments, a FCTR<sub>X</sub> nucleic acid or antisense nucleic acid may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger *et al.*, 1989, *Proc. Natl. Acad. Sci. U.S.A.* 86:6553-6556; Lemaitre *et al.*, 1987, *Proc. Natl. Acad. Sci.* 84:648-652; PCT Publication No. W088/09810) or the blood-brain barrier (see, *e.g.*, PCT Publication No. W089/10134). In addition, oligonucleotides can be modified with hybridization triggered cleavage agents (See, *e.g.*, Krol *et al.*, 1988, *BioTechniques* 6:958-976) or intercalating agents. (See, *e.g.*, Zon, 1988, *Pharm. Res.* 5: 539-549). To this end, the oligonucleotide may be conjugated to another molecule, *e.g.*, a peptide, a hybridization triggered cross-linking agent, a transport agent, a hybridization-triggered cleavage agent, etc.

### FCTR<sub>X</sub> Polypeptides

A FCTR<sub>X</sub> polypeptide of the invention includes a protein whose sequence is provided in SEQ ID NO:2 or 4. The invention also includes a mature form of a FCTR<sub>X</sub> polypeptide, as well as a mutant or variant form of a FCTR<sub>X</sub> polypeptide. In some embodiments, a mutant or variant FCTR<sub>X</sub> includes a protein in which any residues may be changed from the corresponding residue shown in FIG. 1, while still encoding a protein that maintains its FCTR<sub>X</sub>-like activities and physiological functions, or a functional fragment thereof. The invention includes the polypeptides encoded by the variant FCTR<sub>X</sub> nucleic acids described above. In the mutant or variant protein, up to 20% or more of the residues may be so changed.

In general, a FCTR<sub>X</sub> polypeptide variant that preserves FCTR<sub>X</sub> function includes any FCTR<sub>X</sub> polypeptide variant in which residues at a particular position in the sequence have been substituted by other amino acids. A FCTR<sub>X</sub> variant polypeptide also includes a FCTR<sub>X</sub> polypeptide in which an additional residue or residues has been inserted between two residues of the parent protein as well as a protein in which one or more residues have been deleted from

a reference FCTR<sub>X</sub> polypeptide sequence (*e.g.*, SEQ ID NO:2 or SEQ ID NO:4, or a mature form of SEQ ID NO:2 or SEQ ID NO:4). Thus, any amino acid substitution, insertion, or deletion with respect to a reference FCTR<sub>X</sub> polypeptide sequence (*e.g.*, SEQ ID NO:2 or SEQ ID NO:4, or a mature form of SEQ ID NO:2 or SEQ ID NO:4) is encompassed by the invention. In some embodiments, a mutant or variant proteins may include one or more substitutions, insertions, or deletions with respect to a reference FCTR<sub>X</sub> sequence.

The invention also includes isolated FCTR<sub>X</sub> proteins, and biologically active portions thereof, or derivatives, fragments, analogs or homologs thereof. Also provided are polypeptide fragments suitable for use as immunogens to raise anti-FCTR<sub>X</sub> antibodies. In one embodiment, native FCTR<sub>X</sub> proteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, FCTR<sub>X</sub> proteins are produced by recombinant DNA techniques. Alternative to recombinant expression, a FCTR<sub>X</sub> protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

An "isolated" or "purified" protein or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the FCTR<sub>X</sub> protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of a FCTR<sub>X</sub> protein in which the protein is separated from cellular components of the cells from which it is isolated or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations of a FCTR<sub>X</sub> protein having less than about 30% (by dry weight) of non-FCTR<sub>X</sub> protein (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-FCTR<sub>X</sub> protein, still more preferably less than about 10% of non-FCTR<sub>X</sub> protein, and most preferably less than about 5% non-FCTR<sub>X</sub> protein. When the FCTR<sub>X</sub> protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation.

The language "substantially free of chemical precursors or other chemicals" includes preparations of a FCTR<sub>X</sub> protein in which the protein is separated from chemical precursors or other chemicals that are involved in the synthesis of the protein. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of a FCTR<sub>X</sub> protein having less than about 30% (by dry weight) of chemical precursors or non-FCTR<sub>X</sub> polypeptides, more preferably less than about 20% chemical precursors or non-

FCTR<sub>X</sub> polypeptides, still more preferably less than about 10% chemical precursors or non-FCTR<sub>X</sub> polypeptides, and most preferably less than about 5% chemical precursors or non-FCTR<sub>X</sub> polypeptides.

Biologically active portions of a FCTR<sub>X</sub> protein include peptides comprising amino acid sequences sufficiently homologous to or derived from the amino acid sequence of the FCTR<sub>X</sub> protein, *e.g.*, the amino acid sequence shown in SEQ ID NO:2 that include fewer amino acids than the full length FCTR<sub>X</sub> proteins, and exhibit at least one activity of a FCTR<sub>X</sub> protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the FCTR<sub>X</sub> protein. A biologically active portion of a FCTR<sub>X</sub> protein can be a polypeptide which is, for example, 10, 25, 50, 100 or more amino acids in length.

A biologically active portion of a FCTR<sub>X</sub> of the present invention may contain at least one of the above-identified domains conserved among the FCTR<sub>X</sub> family of proteins. Moreover, other biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native FCTR<sub>X</sub> protein.

In some embodiments, the FCTR<sub>X</sub> protein is substantially homologous to any of SEQ ID NOS:2, 4, 6, 8, 10 and 12 and retains the functional activity of the protein of any of SEQ ID NOS:2, 4, 6, 8, 10 and 12, yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail below. Accordingly, in another embodiment, the FCTR<sub>X</sub> protein is a protein that comprises an amino acid sequence at least about 45% homologous, and more preferably about 55, 65, 70, 75, 80, 85, 90, 95, 98 or even 99% homologous to the amino acid sequence of any of SEQ ID NOS:2, 4, 6, 8, 10 and 12 and retains the functional activity of the FCTR<sub>X</sub> proteins of the corresponding polypeptide having the sequence of SEQ ID NOS:2, 4, 6, 8, 10 and 12.

#### **Determining Homology Between Two Or More Sequences**

To determine the percent homology of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in either of the sequences being compared for optimal alignment between the sequences). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are homologous at that position (*i.e.*, as used herein amino acid or nucleic acid "homology" is equivalent to amino acid or nucleic acid "identity").

The nucleic acid sequence homology may be determined as the degree of identity between two sequences. The homology may be determined using computer programs known in the art, such as GAP software provided in the GCG program package. See, *Needleman and Wunsch* 1970 *J Mol Biol* 48: 443-453. Using GCG GAP software with the following settings for nucleic acid sequence comparison: GAP creation penalty of 5.0 and GAP extension penalty of 0.3, the coding region of the analogous nucleic acid sequences referred to above exhibits a degree of identity preferably of at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99%, with the CDS (encoding) part of the DNA sequence shown in SEQ ID NOS:1, 3, 5, 7, 9 and 11. Equivalent software procedures for determining the extent of sequence identity are widely known in the art may be used in the present context.

The term "sequence identity" refers to the degree to which two polynucleotide or polypeptide sequences are identical on a residue-by-residue basis over a particular region of comparison. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over that region of comparison, determining the number of positions at which the identical nucleic acid base (*e.g.*, A, T or U, C, G, or I, in the case of nucleic acids) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the region of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the percentage of sequence identity. The term "substantial identity" as used herein denotes a characteristic of a polynucleotide sequence, wherein the polynucleotide comprises a sequence that has at least 80 percent sequence identity, preferably at least 85 percent identity and often 90 to 95 percent sequence identity, more usually at least 99 percent sequence identity as compared to a reference sequence over a comparison region. The term "percentage of positive residues" is calculated by comparing two optimally aligned sequences over that region of comparison, determining the number of positions at which the identical and conservative amino acid substitutions, as defined above, occur in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the region of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the percentage of positive residues.

#### **Chimeric And Fusion FCTR X Proteins**

The invention also provides FCTR X chimeric or fusion proteins. As used herein, a FCTR X "chimeric protein" or "fusion protein" includes a FCTR X polypeptide operatively linked to a non-FCTR X polypeptide. A "FCTR X polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a FCTR X polypeptide, or a fragment, variant or

derivative thereof, whereas a "non-FCTR<sub>X</sub> polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein that is not substantially homologous to the FCTR<sub>X</sub> protein, *e.g.*, a protein that is different from the FCTR<sub>X</sub> protein and that is derived from the same or a different organism. Thus, within a FCTR<sub>X</sub> fusion protein, the FCTR<sub>X</sub> polypeptide can correspond to all or a portion of a FCTR<sub>X</sub> protein. In one embodiment, a FCTR<sub>X</sub> fusion protein comprises at least one biologically active portion of a FCTR<sub>X</sub> protein. In another embodiment, a FCTR<sub>X</sub> fusion protein comprises at least two biologically active portions of a FCTR<sub>X</sub> protein. Within the fusion protein, the term "operatively linked" is intended to indicate that the FCTR<sub>X</sub> polypeptide and the non-FCTR<sub>X</sub> polypeptide are fused in-frame to each other. The non-FCTR<sub>X</sub> polypeptide can be fused to the N-terminus or C-terminus of the FCTR<sub>X</sub> polypeptide.

For example, in one embodiment a FCTR<sub>X</sub> fusion protein comprises a FCTR<sub>X</sub> polypeptide operably linked to the extracellular domain of a second protein. Such fusion proteins can be further utilized in screening assays for compounds that modulate FCTR<sub>X</sub> activity (such assays are described in detail below).

In another embodiment, the fusion protein is a GST-FCTR<sub>X</sub> fusion protein in which the FCTR<sub>X</sub> sequences are fused to the C-terminus of the GST (*i.e.*, glutathione S-transferase) sequences. Such fusion proteins can facilitate the purification of recombinant FCTR<sub>X</sub>.

In yet another embodiment, the fusion protein is a FCTR<sub>X</sub> protein containing a heterologous signal sequence at its N-terminus. For example, the native FCTR<sub>X</sub> signal sequence can be removed and replaced with a signal sequence from another protein. In certain host cells (*e.g.*, mammalian host cells), expression and/or secretion of the FCTR<sub>X</sub> can be increased through use of a heterologous signal sequence.

In a further embodiment, the fusion protein is a FCTR<sub>X</sub>-immunoglobulin fusion protein in which the FCTR<sub>X</sub> sequences comprising one or more domains are fused to sequences derived from a member of the immunoglobulin protein family. The FCTR<sub>X</sub>-immunoglobulin fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject to inhibit an interaction between a FCTR<sub>X</sub> ligand and a FCTR<sub>X</sub> protein on the surface of a cell, to thereby suppress FCTR<sub>X</sub>-mediated signal transduction *in vivo*. In one example, a contemplated FCTR<sub>X</sub> ligand of the invention is a FCTR<sub>X</sub> receptor. The FCTR<sub>X</sub>-immunoglobulin fusion proteins can be used to modulate the bioavailability of a FCTR<sub>X</sub> cognate ligand. Inhibition of the FCTR<sub>X</sub> ligand/FCTR<sub>X</sub> interaction may be useful therapeutically for both the treatment of proliferative and differentiative disorders, as well as modulating (*e.g.*, promoting or inhibiting) cell survival.



Moreover, the FCTR<sub>X</sub>-immunoglobulin fusion proteins of the invention can be used as immunogens to produce anti-FCTR<sub>X</sub> antibodies in a subject, to purify FCTR<sub>X</sub> ligands, and in screening assays to identify molecules that inhibit the interaction of a FCTR<sub>X</sub> with a FCTR<sub>X</sub> ligand. A FCTR<sub>X</sub> chimeric or fusion protein of the invention can be produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, *e.g.*, by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers that give rise to complementary overhangs between two consecutive gene fragments that can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, Ausubel *et al.* (eds.) CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (*e.g.*, a GST polypeptide). A FCTR<sub>X</sub>-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the FCTR<sub>X</sub> protein.

### **FCTR<sub>X</sub> Agonists And Antagonists**

The present invention also pertains to variants of a FCTR<sub>X</sub> protein that function as either FCTR<sub>X</sub> agonists (mimetics) or as FCTR<sub>X</sub> antagonists. Variants of a FCTR<sub>X</sub> protein can be generated by mutagenesis, *e.g.*, discrete point mutation or truncation of the FCTR<sub>X</sub> protein. An agonist of the FCTR<sub>X</sub> protein can retain substantially the same, or a subset of, the biological activities of the naturally occurring form of the FCTR<sub>X</sub> protein. An antagonist of the FCTR<sub>X</sub> protein can inhibit one or more of the activities of the naturally occurring form of the FCTR<sub>X</sub> protein by, for example, competitively binding to a downstream or upstream member of a cellular signaling cascade which includes the FCTR<sub>X</sub> protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the FCTR<sub>X</sub> protein.

Variants of the FCTR<sub>X</sub> protein that function as either FCTR<sub>X</sub> agonists (mimetics) or as FCTR<sub>X</sub> antagonists can be identified by screening combinatorial libraries of mutants, *e.g.*, truncation mutants, of the FCTR<sub>X</sub> protein for FCTR<sub>X</sub> protein agonist or antagonist activity.

In one embodiment, a variegated library of FCTR<sub>X</sub> variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of FCTR<sub>X</sub> variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential FCTR<sub>X</sub> sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (e.g., for phage display) containing the set of FCTR<sub>X</sub> sequences therein. There are a variety of methods which can be used to produce libraries of potential FCTR<sub>X</sub> variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential FCTR<sub>X</sub> variant sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (see, e.g., Narang (1983) *Tetrahedron* 39:3; Itakura *et al.* (1984) *Annu Rev Biochem* 53:323; Itakura *et al.* (1984) *Science* 198:1056; Ike *et al.* (1983) *Nucl Acid Res* 11:477).

### Polypeptide Libraries

In addition, libraries of fragments of the FCTR<sub>X</sub> protein coding sequence can be used to generate a variegated population of growth promoter fragments for screening and subsequent selection of variants of a FCTR<sub>X</sub> protein. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of a FCTR<sub>X</sub> coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA that can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes N-terminal and internal fragments of various sizes of the FCTR<sub>X</sub> protein.

Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of FCTR<sub>X</sub> proteins. The most widely used techniques, which are amenable to high throughput analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the

combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a new technique that enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify FCTR<sub>X</sub> variants (Arkin and Yourvan (1992) PNAS 89:7811-7815; Delgrave *et al.* (1993) Protein Engineering 6:327-331).

### Anti-FCTR<sub>X</sub> Antibodies

The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin (Ig) molecules, *i.e.*, molecules that contain an antigen binding site that specifically binds (immunoreacts with) an antigen. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, F<sub>ab</sub>, F<sub>ab</sub>' and F<sub>(ab')<sub>2</sub></sub> fragments, and an F<sub>ab</sub> expression library. In general, antibody molecules obtained from humans relates to any of the classes IgG, IgM, IgA, IgE and IgD, which differ from one another by the nature of the heavy chain present in the molecule. Certain classes have subclasses as well, such as IgG<sub>1</sub>, IgG<sub>2</sub>, and others. Furthermore, in humans, the light chain may be a kappa chain or a lambda chain. Reference herein to antibodies includes a reference to all such classes, subclasses and types of human antibody species.

An isolated protein of the invention intended to serve as an antigen, or a portion or fragment thereof, can be used as an immunogen to generate antibodies that immunospecifically bind the antigen, using standard techniques for polyclonal and monoclonal antibody preparation. The full-length protein can be used or, alternatively, the invention provides antigenic peptide fragments of the antigen for use as immunogens. An antigenic peptide fragment comprises at least 6 amino acid residues of the amino acid sequence of the full length protein, such as an amino acid sequence shown in SEQ ID NOS:2, 4, 6, 8, 10 and 12, and encompasses an epitope thereof such that an antibody raised against the peptide forms a specific immune complex with the full length protein or with any fragment that contains the epitope. Preferably, the antigenic peptide comprises at least 10 amino acid residues, or at least 15 amino acid residues, or at least 20 amino acid residues, or at least 30 amino acid residues. Preferred epitopes encompassed by the antigenic peptide are regions of the protein that are located on its surface; commonly these are hydrophilic regions.

In certain embodiments of the invention, at least one epitope encompassed by the antigenic peptide is a region of the FCTR<sub>X</sub> that is located on the surface of the protein, *e.g.*, a hydrophilic region. A hydrophobicity analysis of the human FCTR<sub>X</sub> protein sequence will indicate which regions of a FCTR<sub>X</sub> polypeptide are particularly hydrophilic and, therefore, are

likely to encode surface residues useful for targeting antibody production. As a means for targeting antibody production, hydropathy plots showing regions of hydrophilicity and hydrophobicity may be generated by any method well known in the art, including, for example, the Kyte Doolittle or the Hopp Woods methods, either with or without Fourier transformation. See, *e.g.*, Hopp and Woods, 1981, *Proc. Nat. Acad. Sci. USA* 78: 3824-3828; Kyte and Doolittle 1982, *J. Mol. Biol.* 157: 105-142, each incorporated herein by reference in their entirety. Antibodies that are specific for one or more domains within an antigenic protein, or derivatives, fragments, analogs or homologs thereof, are also provided herein.

A protein of the invention, or a derivative, fragment, analog, homolog or ortholog thereof, may be utilized as an immunogen in the generation of antibodies that immunospecifically bind these protein components.

Various procedures known within the art may be used for the production of polyclonal or monoclonal antibodies directed against a protein of the invention, or against derivatives, fragments, analogs homologs or orthologs thereof (see, for example, *Antibodies: A Laboratory Manual*, Harlow E, and Lane D, 1988, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, incorporated herein by reference). Some of these antibodies are discussed below.

### **Polyclonal Antibodies**

For the production of polyclonal antibodies, various suitable host animals (*e.g.*, rabbit, goat, mouse or other mammal) may be immunized by one or more injections with the native protein, a synthetic variant thereof, or a derivative of the foregoing. An appropriate immunogenic preparation can contain, for example, the naturally occurring immunogenic protein, a chemically synthesized polypeptide representing the immunogenic protein, or a recombinantly expressed immunogenic protein. Furthermore, the protein may be conjugated to a second protein known to be immunogenic in the mammal being immunized. Examples of such immunogenic proteins include but are not limited to keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, and soybean trypsin inhibitor. The preparation can further include an adjuvant. Various adjuvants used to increase the immunological response include, but are not limited to, Freund's (complete and incomplete), mineral gels (*e.g.*, aluminum hydroxide), surface active substances (*e.g.*, lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, dinitrophenol, etc.), adjuvants usable in humans such as Bacille Calmette-Guerin and *Corynebacterium parvum*, or similar immunostimulatory agents. Additional examples of adjuvants which can be employed include MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate).

The polyclonal antibody molecules directed against the immunogenic protein can be isolated from the mammal (e.g., from the blood) and further purified by well known techniques, such as affinity chromatography using protein A or protein G, which provide primarily the IgG fraction of immune serum. Subsequently, or alternatively, the specific antigen which is the target of the immunoglobulin sought, or an epitope thereof, may be immobilized on a column to purify the immune specific antibody by immunoaffinity chromatography. Purification of immunoglobulins is discussed, for example, by D. Wilkinson (The Scientist, published by The Scientist, Inc., Philadelphia PA, Vol. 14, No. 8 (April 17, 2000), pp. 25-28).

### **Monoclonal Antibodies**

The term "monoclonal antibody" (MAb) or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one molecular species of antibody molecule consisting of a unique light chain gene product and a unique heavy chain gene product. In particular, the complementarity determining regions (CDRs) of the monoclonal antibody are identical in all the molecules of the population. MAbs thus contain an antigen binding site capable of immunoreacting with a particular epitope of the antigen characterized by a unique binding affinity for it.

Monoclonal antibodies can be prepared using hybridoma methods, such as those described in the art. See, e.g., Kohler and Milstein, 1975 Nature, 256:495. In a hybridoma method, a mouse, hamster, or other appropriate host animal, is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes can be immunized *in vitro*.

The immunizing agent will typically include the protein antigen, a fragment thereof or a fusion protein thereof. Generally, either peripheral blood lymphocytes are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, MONOCLONAL ANTIBODIES: PRINCIPLES AND PRACTICE, Academic Press, (1986) pp. 59-103). Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells can be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine

phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which substances prevent the growth of HGPRT-deficient cells.

Preferred immortalized cell lines are those that fuse efficiently, support stable high level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. More preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, California and the American Type Culture Collection, Manassas, Virginia. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies. See, *e.g.* Kozbor 1984 *J. Immunol.*, 133:3001; Brodeur *et al.* MONOCLONAL ANTIBODY PRODUCTION TECHNIQUES AND APPLICATIONS, Marcel Dekker, Inc., New York, (1987) pp. 51-63.

The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by the hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). Such techniques and assays are known in the art. The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis. See, *e.g.* Munson and Pollard 1980 *Anal. Biochem.* 107: 220. It is an objective, especially important in therapeutic applications of monoclonal antibodies, to identify antibodies having a high degree of specificity and a high binding affinity for the target antigen.

After the desired hybridoma cells are identified, the clones can be subcloned by limiting dilution procedures and grown by standard methods (Goding, 1986). Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640 medium. Alternatively, the hybridoma cells can be grown *in vivo* as ascites in a mammal.

The monoclonal antibodies secreted by the subclones can be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

The monoclonal antibodies can also be made by recombinant DNA methods, such as those described in U.S. Patent No. 4,816,577. DNA encoding the monoclonal antibodies of the invention can be readily isolated and sequenced using conventional procedures (*e.g.*, by

using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA can be placed into expression vectors, which are then transfected into host cells such as simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also can be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences (U.S. Patent No. 4,816,567; Morrison, *Nature* 368, 812-13 (1994)) or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention, or can be substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

#### **Humanized Antibodies**

The antibodies directed against the protein antigens of the invention can further comprise humanized antibodies or human antibodies. These antibodies are suitable for administration to humans without engendering an immune response by the human against the administered immunoglobulin. Humanized forms of antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')<sub>2</sub> or other antigen-binding subsequences of antibodies) that are principally comprised of the sequence of a human immunoglobulin, and contain minimal sequence derived from a non-human immunoglobulin. Humanization can be performed following the method of Winter and co-workers (Jones *et al.*, *Nature*, 321:522-525 (1986); Riechmann *et al.*, *Nature*, 332:323-327 (1988); Verhoeyen *et al.*, *Science*, 239:1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. (See also U.S. Patent No. 5,225,539.) In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies can also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that

of a human immunoglobulin (Jones *et al.*, 1986; Riechmann *et al.*, 1988; and Presta, Curr. Op. Struct. Biol., 2:593-596 (1992)).

### Human Antibodies

Fully human antibodies essentially relate to antibody molecules in which the entire sequence of both the light chain and the heavy chain, including the CDRs, arise from human genes. Such antibodies are termed "human antibodies", or "fully human antibodies" herein. Human monoclonal antibodies can be prepared by the trioma technique; the human B-cell hybridoma technique (see Kozbor, *et al.*, 1983 *Immunol Today* 4: 72) and the EBV hybridoma technique to produce human monoclonal antibodies (see Cole, *et al.*, 1985 In: *MONOCLONAL ANTIBODIES AND CANCER THERAPY*, Alan R. Liss, Inc., pp. 77-96). Human monoclonal antibodies may be utilized in the practice of the present invention and may be produced by using human hybridomas (see Cote, *et al.*, 1983. *Proc Natl Acad Sci USA* 80: 2026-2030) or by transforming human B-cells with Epstein Barr Virus *in vitro* (see Cole, *et al.*, 1985 In: *MONOCLONAL ANTIBODIES AND CANCER THERAPY*, Alan R. Liss, Inc., pp. 77-96).

In addition, human antibodies can also be produced using additional techniques, including phage display libraries (Hoogenboom and Winter, J. Mol. Biol., 227:381 (1991); Marks *et al.*, J. Mol. Biol., 222:581 (1991)). Similarly, human antibodies can be made by introducing human immunoglobulin loci into transgenic animals, *e.g.*, mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in Marks *et al.* (Bio/Technology 10, 779-783 (1992)); Lonberg *et al.* (Nature 368 856-859 (1994)); Morrison (Nature 368, 812-13 (1994)); Fishwild *et al.*, (Nature Biotechnology 14, 845-51 (1996)); Neuberger (Nature Biotechnology 14, 826 (1996)); and Lonberg and Huszar (Intern. Rev. Immunol. 13 65-93 (1995)).

Human antibodies may additionally be produced using transgenic nonhuman animals which are modified so as to produce fully human antibodies rather than the animal's endogenous antibodies in response to challenge by an antigen. (See publication WO 94/02602). The endogenous genes encoding the heavy and light immunoglobulin chains in the nonhuman host have been incapacitated, and active loci encoding human heavy and light chain immunoglobulins are inserted into the host's genome. The human genes are incorporated, for example, using yeast artificial chromosomes containing the requisite human DNA segments. An animal which provides all the desired modifications is then obtained as progeny by



crossbreeding intermediate transgenic animals containing fewer than the full complement of the modifications. The preferred embodiment of such a nonhuman animal is a mouse, and is termed the Xenomouse<sup>TM</sup> as disclosed in PCT publications WO 96/33735 and WO 96/34096. This animal produces B cells which secrete fully human immunoglobulins. The antibodies can be obtained directly from the animal after immunization with an immunogen of interest, as, for example, a preparation of a polyclonal antibody, or alternatively from immortalized B cells derived from the animal, such as hybridomas producing monoclonal antibodies. Additionally, the genes encoding the immunoglobulins with human variable regions can be recovered and expressed to obtain the antibodies directly, or can be further modified to obtain analogs of antibodies such as, for example, single chain Fv molecules.

An example of a method of producing a nonhuman host, exemplified as a mouse, lacking expression of an endogenous immunoglobulin heavy chain is disclosed in U.S. Patent No. 5,939,598. It can be obtained by a method including deleting the J segment genes from at least one endogenous heavy chain locus in an embryonic stem cell to prevent rearrangement of the locus and to prevent formation of a transcript of a rearranged immunoglobulin heavy chain locus, the deletion being effected by a targeting vector containing a gene encoding a selectable marker; and producing from the embryonic stem cell a transgenic mouse whose somatic and germ cells contain the gene encoding the selectable marker.

A method for producing an antibody of interest, such as a human antibody, is disclosed in U.S. Patent No. 5,916,771. It includes introducing an expression vector that contains a nucleotide sequence encoding a heavy chain into one mammalian host cell in culture, introducing an expression vector containing a nucleotide sequence encoding a light chain into another mammalian host cell, and fusing the two cells to form a hybrid cell. The hybrid cell expresses an antibody containing the heavy chain and the light chain.

In a further improvement on this procedure, a method for identifying a clinically relevant epitope on an immunogen, and a correlative method for selecting an antibody that binds immunospecifically to the relevant epitope with high affinity, are disclosed in PCT publication WO 99/53049.

#### **F<sub>ab</sub> Fragments and Single Chain Antibodies**

Techniques can be adapted for the production of single-chain antibodies specific to an antigenic protein of the invention (see *e.g.*, U.S. Patent No. 4,946,778). In addition, methods can be adapted for the construction of F<sub>ab</sub> expression libraries (see *e.g.*, Huse, *et al.*, 1989 Science 246: 1275-1281) to allow rapid and effective identification of monoclonal F<sub>ab</sub> fragments with the desired specificity for a protein or derivatives, fragments, analogs or

homologs thereof. Antibody fragments that contain the idiotypes to a protein antigen may be produced by techniques known in the art including, but not limited to: (i) an  $F_{(ab)2}$  fragment produced by pepsin digestion of an antibody molecule; (ii) an  $F_{ab}$  fragment generated by reducing the disulfide bridges of an  $F_{(ab)2}$  fragment; (iii) an  $F_{ab}$  fragment generated by the treatment of the antibody molecule with papain and a reducing agent and (iv)  $F_v$  fragments.

### **Bispecific Antibodies**

Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for an antigenic protein of the invention. The second binding target is any other antigen, and advantageously is a cell-surface protein or receptor or receptor subunit.

Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy-chain/light-chain pairs, where the two heavy chains have different specificities (Milstein and Cuello, Nature, 305:537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule is usually accomplished by affinity chromatography steps. Similar procedures are disclosed in WO 93/08829, published 13 May 1993, and in Traunecker *et al.*, EMBO J., 10:3655-3659 (1991).

Antibody variable domains with the desired binding specificities (antibody-antigen combining sites) can be fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light-chain binding present in at least one of the fusions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. For further details of generating bispecific antibodies see, for example, Suresh *et al.*, Methods in Enzymology, 121:210 (1986).

According to another approach described in WO 96/27011, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface comprises at least a part of the CH3 region of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (*e.g.* tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the

large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (e.g. alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

Bispecific antibodies can be prepared as full length antibodies or antibody fragments (e.g. F(ab')<sub>2</sub> bispecific antibodies). Techniques for generating bispecific antibodies from antibody fragments have been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan *et al.*, Science 229:81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate F(ab')<sub>2</sub> fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

Additionally, Fab' fragments can be directly recovered from *E. coli* and chemically coupled to form bispecific antibodies. Shalaby *et al.*, J. Exp. Med. 175:217-225 (1992) describe the production of a fully humanized bispecific antibody F(ab')<sub>2</sub> molecule. Each Fab' fragment was separately secreted from *E. coli* and subjected to directed chemical coupling *in vitro* to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the ErbB2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny *et al.*, J. Immunol. 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger *et al.*, Proc. Natl. Acad. Sci. USA 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain (V<sub>H</sub>) connected to a light-chain variable domain (V<sub>L</sub>) by a linker which is too short to allow pairing between the two domains on the same chain.

Accordingly, the V<sub>H</sub> and V<sub>L</sub> domains of one fragment are forced to pair with the complementary V<sub>L</sub> and V<sub>H</sub> domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See, Gruber *et al.*, J. Immunol. 152:5368 (1994).

Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tutt *et al.*, J. Immunol. 147:60 (1991).

Exemplary bispecific antibodies can bind to two different epitopes, at least one of which originates in the protein antigen of the invention. Alternatively, an anti-antigenic arm of an immunoglobulin molecule can be combined with an arm which binds to a triggering molecule on a leukocyte such as a T-cell receptor molecule (*e.g.* CD2, CD3, CD28, or B7), or Fc receptors for IgG (FcγR), such as FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16) so as to focus cellular defense mechanisms to the cell expressing the particular antigen. Bispecific antibodies can also be used to direct cytotoxic agents to cells which express a particular antigen. These antibodies possess an antigen-binding arm and an arm which binds a cytotoxic agent or a radionuclide chelator, such as EOTUBE, DPTA, DOTA, or TETA. Another bispecific antibody of interest binds the protein antigen described herein and further binds tissue factor (TF).

### **Heteroconjugate Antibodies**

Heteroconjugate antibodies are also within the scope of the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. Patent No. 4,676,980), and for treatment of HIV infection (WO 91/00360; WO 92/200373; EP 03089). It is contemplated that the antibodies can be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins can be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate and those disclosed, for example, in U.S. Patent No. 4,676,980.

### **Effector Function Engineering**

It can be desirable to modify the antibody of the invention with respect to effector function, so as to enhance, *e.g.*, the effectiveness of the antibody in treating cancer. For example, cysteine residue(s) can be introduced into the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated can have

improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron *et al.*, J. Exp Med., **176**: 1191-1195 (1992) and Shopes, J. Immunol., **148**: 2918-2922 (1992). Homodimeric antibodies with enhanced anti-tumor activity can also be prepared using heterobifunctional cross-linkers as described in Wolff *et al.* Cancer Research, **53**: 2560-2565 (1993). Alternatively, an antibody can be engineered that has dual Fc regions and can thereby have enhanced complement lysis and ADCC capabilities. See Stevenson *et al.*, Anti-Cancer Drug Design, **3**: 219-230 (1989).

### Immunoconjugates

The invention also pertains to immunoconjugates comprising an antibody conjugated to a cytotoxic agent such as a chemotherapeutic agent, toxin (*e.g.*, an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (*i.e.*, a radioconjugate).

Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above. Enzymatically active toxins and fragments thereof that can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolacca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes. A variety of radionuclides are available for the production of radioconjugated antibodies. Examples include  $^{212}\text{Bi}$ ,  $^{131}\text{I}$ ,  $^{131}\text{In}$ ,  $^{90}\text{Y}$ , and  $^{186}\text{Re}$ .

Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein-coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP); iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta *et al.*, Science, **238**: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026.

In another embodiment, the antibody can be conjugated to a "receptor" (such as streptavidin) for utilization in tumor pretargeting wherein the antibody-receptor conjugate is

administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (*e.g.*, avidin) that is in turn conjugated to a cytotoxic agent.

### **Immunoliposomes**

The antibodies disclosed herein can also be formulated as immunoliposomes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein *et al.*, Proc. Natl. Acad. Sci. USA, **82**: 3688 (1985); Hwang *et al.*, Proc. Natl. Acad. Sci. USA, **77**: 4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556.

Particularly useful liposomes can be generated by the reverse-phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol, and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the present invention can be conjugated to the liposomes as described in Martin *et al.*, J. Biol. Chem., **257**: 286-288 (1982) via a disulfide-interchange reaction. A chemotherapeutic agent (such as Doxorubicin) is optionally contained within the liposome. See Gabizon *et al.*, J. National Cancer Inst., **81**(19): 1484 (1989).

### **Diagnostic Applications of Antibodies Directed Against the Proteins of the Invention**

Antibodies directed against a protein of the invention may be used in methods known within the art relating to the localization and/or quantitation of the protein (*e.g.*, for use in measuring levels of the protein within appropriate physiological samples, for use in diagnostic methods, for use in imaging the protein, and the like). In a given embodiment, antibodies against the proteins, or derivatives, fragments, analogs or homologs thereof, that contain the antigen binding domain, are utilized as pharmacologically-active compounds (see below).

An antibody specific for a protein of the invention can be used to isolate the protein by standard techniques, such as immunoaffinity chromatography or immunoprecipitation. Such an antibody can facilitate the purification of the natural protein antigen from cells and of recombinantly produced antigen expressed in host cells. Moreover, such an antibody can be used to detect the antigenic protein (*e.g.*, in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the antigenic protein. Antibodies directed against the protein can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, *e.g.*, to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (*i.e.*, physically linking) the antibody to a

detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase,  $\beta$ -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{35}\text{S}$  or  $^3\text{H}$ .

### Pharmaceutical Compositions of Antibodies

Antibodies specifically binding a protein of the invention, as well as other molecules identified by the screening assays disclosed herein, can be administered for the treatment of various disorders in the form of pharmaceutical compositions. Principles and considerations involved in preparing such compositions, as well as guidance in the choice of components are provided, for example, in Remington : THE SCIENCE AND PRACTICE OF PHARMACY 19th ed. (Alfonso R. Gennaro, *et al.*, editors) Mack Pub. Co., Easton, Pa. 1995; DRUG ABSORPTION ENHANCEMENT: CONCEPTS, POSSIBILITIES, LIMITATIONS, AND TRENDS, Harwood Academic Publishers, Langhorne, Pa., 1994; and PEPTIDE AND PROTEIN DRUG DELIVERY (Advances In Parenteral Sciences, Vol. 4), 1991, M. Dekker, New York.

If the antigenic protein is intracellular and whole antibodies are used as inhibitors, internalizing antibodies are preferred. However, liposomes can also be used to deliver the antibody, or an antibody fragment, into cells. Where antibody fragments are used, the smallest inhibitory fragment that specifically binds to the binding domain of the target protein is preferred. For example, based upon the variable-region sequences of an antibody, peptide molecules can be designed that retain the ability to bind the target protein sequence. Such peptides can be synthesized chemically and/or produced by recombinant DNA technology. See, *e.g.*, Marasco *et al.*, Proc. Natl. Acad. Sci. USA, 90: 7889-7893 (1993). The formulation herein can also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Alternatively, or in addition, the composition can comprise an agent that enhances its function, such as, for example, a cytotoxic agent, cytokine, chemotherapeutic agent, or growth-inhibitory agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

The active ingredients can also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylemethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles, and nanocapsules) or in macroemulsions.

The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

### **Antibody Therapeutics**

Antibodies of the invention, including polyclonal, monoclonal, humanized and fully human antibodies, may be used as therapeutic agents. Such agents will generally be employed to treat or prevent a disease or pathology in a subject. An antibody preparation, preferably one having high specificity and high affinity for its target antigen, is administered to the subject and will generally have an effect due to its binding with the target. Such an effect may be one of two kinds, depending on the specific nature of the interaction between the given antibody molecule and the target antigen in question. In the first instance, administration of the antibody may abrogate or inhibit the binding of the target with an endogenous ligand to which it naturally binds. In this case, the antibody binds to the target and masks a binding site of the naturally occurring ligand, wherein the ligand serves as an effector molecule. Thus the receptor mediates a signal transduction pathway for which ligand is responsible.

Alternatively, the effect may be one in which the antibody elicits a physiological result by virtue of binding to an effector binding site on the target molecule. In this case the target, a receptor having an endogenous ligand which may be absent or defective in the disease or pathology, binds the antibody as a surrogate effector ligand, initiating a receptor-based signal transduction event by the receptor.

A therapeutically effective amount of an antibody of the invention relates generally to the amount needed to achieve a therapeutic objective. As noted above, this may be a binding interaction between the antibody and its target antigen that, in certain cases, interferes with the functioning of the target, and in other cases, promotes a physiological response. The amount required to be administered will furthermore depend on the binding affinity of the antibody for its specific antigen, and will also depend on the rate at which an administered antibody is depleted from the free volume of the subject to which it is administered. Common ranges for therapeutically effective dosing of an antibody or antibody fragment of the invention may be, by way of nonlimiting example, from about 0.1 mg/kg body weight to about 50 mg/kg body



weight. Common dosing frequencies may range, for example, from twice daily to once a week.

### **FCTRX Recombinant Vectors and Host Cells**

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding a FCTRX protein, or derivatives, fragments, analogs or homologs thereof. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, that is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner that allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel; GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cell

and those that direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (*e.g.*, FCTR<sub>X</sub> proteins, mutant forms of the FCTR<sub>X</sub>, fusion proteins, etc.).

The recombinant expression vectors of the invention can be designed for expression of a FCTR<sub>X</sub> nucleic acid in prokaryotic or eukaryotic cells. For example, the FCTR<sub>X</sub> can be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example, using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: (1) to increase expression of recombinant protein; (2) to increase the solubility of the recombinant protein; and (3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often a proteolytic cleavage site is introduced in fusion expression vectors at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith and Johnson (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, Mass.) and pRIT5 (Pharmacia, Piscataway, N.J.) that fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amrann *et al.*, (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.*, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 60-89).

One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant

protein. See, Gottesman, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 119-128. Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (Wada *et al.*, (1992) *Nucleic Acids Res.* 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the FCTR<sub>X</sub> expression vector is a yeast expression vector. Examples of vectors for expression in yeast *S. cerevisiae* include pYepSec1 (Baldari, *et al.*, (1987) *EMBO J* 6:229-234), pMFa (Kurjan and Herskowitz, (1982) *Cell* 30:933-943), pJRY88 (Schultz *et al.*, (1987) *Gene* 54:113-123), pYES2 (Invitrogen Corporation, San Diego, Calif.), and picZ (InVitrogen Corp, San Diego, Calif.).

Alternatively, the FCTR<sub>X</sub> nucleic acid can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (*e.g.*, SF9 cells) include the pAc series (Smith *et al.* (1983) *Mol Cell Biol* 3:2156-2165) and the pVL series (Lucklow and Summers (1989) *Virology* 170:31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed (1987) *Nature* 329:840) and pMT2PC (Kaufman *et al.* (1987) *EMBO J* 6: 187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells. See, *e.g.*, Chapters 16 and 17 of Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert *et al.* (1987) *Genes Dev* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv Immunol* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J* 8:729-733) and immunoglobulins (Banerji *et al.* (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (*e.g.*, the neurofilament promoter; Byrne and

Ruddle (1989) *PNAS* 86:5473-5477), pancreas-specific promoters (Edlund *et al.* (1985) *Science* 230:912-916), and mammary gland-specific promoters (*e.g.*, milk whey promoter; U.S. Pat. No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, *e.g.*, the murine *hox* promoters (Kessel and Gruss (1990) *Science* 249:374-379) and the  $\alpha$ -fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev* 3:537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner that allows for expression (by transcription of the DNA molecule) of an RNA molecule that is antisense to a FCTR<sub>X</sub> mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen that direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen that direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes see Weintraub *et al.*, "Antisense RNA as a molecular tool for genetic analysis," Reviews--Trends in Genetics, Vol. 1(1) 1986.

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, the FCTR<sub>X</sub> protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing

foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.* (MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Various selectable markers include those that confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding the growth promoter or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) the FCTR<sub>X</sub> protein. Accordingly, the invention further provides methods for producing the FCTR<sub>X</sub> protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding the FCTR<sub>X</sub> polypeptide has been introduced) in a suitable medium such that the FCTR<sub>X</sub> protein is produced. In another embodiment, the method further comprises isolating the FCTR<sub>X</sub> from the medium or the host cell.

### **Transgenic Animals**

The host cells of the invention can also be used to produce nonhuman transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which FCTR<sub>X</sub>-coding sequences have been introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous FCTR<sub>X</sub> sequences have been introduced into their genome or homologous recombinant animals in which endogenous FCTR<sub>X</sub> sequences have been altered. Such animals are useful for studying the function and/or activity of the FCTR<sub>X</sub> sequences and for identifying and/or evaluating modulators of FCTR<sub>X</sub> activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of

transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, etc. A transgene is exogenous DNA that is integrated into the genome of a cell from which a transgenic animal develops and that remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which the endogenous FCTR<sub>X</sub> gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an embryonic cell of the animal, prior to development of the animal.

A transgenic animal of the invention can be created by introducing FCTR<sub>X</sub>-encoding nucleic acid into the nucleus of a pronuclei of a fertilized oocyte, *e.g.*, by microinjection, retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. The human FCTR<sub>X</sub> DNA sequence of SEQ ID NOS:1, 3, 5, 7, 9 and 11 can be introduced as a transgene into the genome of a non-human animal. Alternatively, a nonhuman homologue of the human FCTR<sub>X</sub> gene, such as a mouse FCTR<sub>X</sub> gene, can be isolated based on hybridization to the human FCTR<sub>X</sub> cDNA (described further above) and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to the FCTR<sub>X</sub> transgene to direct expression of FCTR<sub>X</sub> protein to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Pat. Nos. 4,736,866; 4,870,009; and 4,873,191; and Hogan et al., 1986, In: *MANIPULATING THE MOUSE EMBRYO*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of the FCTR<sub>X</sub> transgene in its genome and expression of FCTR<sub>X</sub> mRNA in tissues or cells of the animal. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding a FCTR<sub>X</sub> can further be bred to other transgenic animals carrying other transgenes.

To create a homologous recombinant animal, a vector is prepared which contains at least a portion of the FCTR<sub>X</sub> gene into which a deletion, addition or substitution has been introduced to the gene, *e.g.*, functionally disrupt, the FCTR<sub>X</sub> gene. The FCTR<sub>X</sub> gene can be a human gene, *e.g.*, SEQ ID NOS:1, 3, 5, 7, 9 and 11), but more preferably, is a non-human homolog of a human FCTR<sub>X</sub> gene. For example, a mouse homologue of human

FCTR<sub>X</sub> gene of SEQ ID NOS:1, 3, 5, 7, 9 and 11 can be used to construct a homologous recombination vector suitable for altering an endogenous FCTR<sub>X</sub> gene in the mouse genome. In one embodiment, the vector is designed such that, upon homologous recombination, the endogenous FCTR<sub>X</sub> gene is functionally disrupted (*i.e.*, no longer encodes a functional protein; also referred to as a "knock out" vector).

Alternatively, the vector can be designed such that, upon homologous recombination, the endogenous FCTR<sub>X</sub> gene is mutated or otherwise altered but still encodes functional protein (*e.g.*, the upstream regulatory region can be altered to thereby alter the expression of the endogenous FCTR<sub>X</sub> protein). In the homologous recombination vector, the altered portion of the FCTR<sub>X</sub> gene is flanked at its 5' and 3' ends by additional nucleic acid of the FCTR<sub>X</sub> gene to allow for homologous recombination to occur between the exogenous FCTR<sub>X</sub> protein gene carried by the vector and an endogenous FCTR<sub>X</sub> protein gene in an embryonic stem cell. The additional flanking FCTR<sub>X</sub> protein nucleic acid is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5' and 3' ends) are included in the vector. See *e.g.*, Thomas *et al.* (1987) *Cell* 51:503 for a description of homologous recombination vectors. The vector is introduced into an embryonic stem cell line (*e.g.*, by electroporation) and cells in which the introduced FCTR<sub>X</sub> protein gene has homologously recombined with the endogenous FCTR<sub>X</sub> protein gene are selected (see *e.g.*, Li *et al.* (1992) *Cell* 69:915).

The selected cells are then injected into a blastocyst of an animal (*e.g.*, a mouse) to form aggregation chimeras. See *e.g.*, Bradley 1987, In: TERATOCARCINOMAS AND EMBRYONIC STEM CELLS: A PRACTICAL APPROACH, Robertson, ed. IRL, Oxford, pp. 113-152. A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination vectors and homologous recombinant animals are described further in Bradley (1991) *Curr Opin Biotechnol* 2:823-829; PCT International Publication Nos.: WO 90/1184; WO 91/01140; WO 92/0968; and WO 93/04169.

In another embodiment, transgenic non-humans animals can be produced that contain selected systems that allow for regulated expression of the transgene. One example of such a system is the cre/loxP recombinase system of bacteriophage P1. For a description of the cre/loxP recombinase system, see, *e.g.*, Lakso *et al.* (1992) *PNAS* 89:6232-6236. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae*

(O'Gorman *et al.* (1991) *Science* 251:181-185. If a cre/loxP recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the Cre recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, *e.g.*, by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut *et al.* (1997) *Nature* 385:810-813. In brief, a cell, *e.g.*, a somatic cell, from the transgenic animal can be isolated and induced to exit the growth cycle and enter G<sub>0</sub> phase. The quiescent cell can then be fused, *e.g.*, through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such that it develops to morula or blastocyte and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell, *e.g.*, the somatic cell, is isolated.

### Pharmaceutical Compositions

The FCTR<sub>X</sub> nucleic acid molecules, FCTR<sub>X</sub> proteins, and anti-FCTR<sub>X</sub> antibodies of the invention, and derivatives, fragments, analogs and homologs thereof are designated "active compounds" or "Therapeutics" herein. Additionally, low molecular weight compounds which have the property that they either bind to the FCTR<sub>X</sub> nucleic acid molecules, the FCTR<sub>X</sub> proteins, and the anti-FCTR<sub>X</sub> antibodies of the invention, and derivatives, fragments, analogs and homologs thereof, or induce pharmacological agonist or antagonist responses commonly ascribed to a FCTR<sub>X</sub> nucleic acid molecule, a FCTR<sub>X</sub> protein, and derivatives, fragments, analogs and homologs thereof, are also termed "active compounds" or "Therapeutics" herein. These Therapeutics can be incorporated into pharmaceutical compositions suitable for administration to a subject. Such compositions typically comprise the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier.

As used herein, "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Suitable carriers are described in the most recent edition of Remington's Pharmaceutical Sciences, a standard reference text in the field, which is incorporated herein by reference. Preferred examples of such carriers or diluents include, but are not limited to, water, saline, Ringer's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-



aqueous vehicles such as fixed oils may also be used. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates, and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride in the

composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (*e.g.*, a FCTR<sub>X</sub> protein or anti-FCTR<sub>X</sub> protein antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays

or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Pat. No. 4,522,811.

Sustained-release preparations can be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, *e.g.*, films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT<sup>TM</sup> (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release pharmaceutical active agents over shorter time periods.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent

on the unique characteristics of the active compound and the particular therapeutic effect to be achieved.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by any of a number of routes, *e.g.*, as described in U.S. Patent Nos. 5,703,055. Delivery can thus also include, *e.g.*, intravenous injection, local administration (see U.S. Pat. No. 5,328,470) or stereotactic injection (see *e.g.*, Chen *et al.* (1994) *PNAS* 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells that produce the gene delivery system.

The pharmaceutical compositions can be included in a kit, *e.g.*, in a container, pack, or dispenser together with instructions for administration.

Also within the invention is the use of a therapeutic in the manufacture of a medicament for treating a syndrome associated with a human disease, the disease selected from a FCTR<sub>X</sub>-associated disorder, wherein said therapeutic is selected from the group consisting of a FCTR<sub>X</sub> polypeptide, a FCTR<sub>X</sub> nucleic acid, and an anti-FCTR<sub>X</sub> antibody.

#### **Additional Uses and Methods of the Invention**

The nucleic acid molecules, proteins, protein homologues, and antibodies described herein can be used in one or more of the following methods: (a) screening assays; (b) detection assays (*e.g.*, chromosomal mapping, cell and tissue typing, forensic biology), (c) predictive medicine (*e.g.*, diagnostic assays, prognostic assays, monitoring clinical trials, and pharmacogenomics); and (d) methods of treatment (*e.g.*, therapeutic and prophylactic).

The isolated nucleic acid molecules of the invention can be used to express a FCTR<sub>X</sub> protein (*e.g.*, via a recombinant expression vector in a host cell in gene therapy applications), to detect a FCTR<sub>X</sub> mRNA (*e.g.*, in a biological sample) or a genetic lesion in a FCTR<sub>X</sub> gene, and to modulate FCTR<sub>X</sub> activity, as described further below. In addition, the FCTR<sub>X</sub> proteins can be used to screen drugs or compounds that modulate the FCTR<sub>X</sub> activity or expression as well as to treat disorders characterized by insufficient or excessive production of the FCTR<sub>X</sub> protein, for example proliferative or differentiative disorders, or production of the FCTR<sub>X</sub> protein forms that have decreased or aberrant activity compared to the FCTR<sub>X</sub> wild type protein. In addition, the anti-FCTR<sub>X</sub> antibodies of the invention can be used to detect and isolate FCTR<sub>X</sub> proteins and modulate FCTR<sub>X</sub> activity.

This invention further pertains to novel agents identified by the above described screening assays and uses thereof for treatments as described herein.

### Screening Assays

The invention provides methods (also referred to herein as "screening assays") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, proteins, polypeptides, nucleic acids or polynucleotides, peptides, peptidomimetics, small molecules including agonists or antagonists, or other drugs) that bind to FCTR<sub>X</sub> proteins or have a stimulatory or inhibitory effect on, for example, FCTR<sub>X</sub> expression or FCTR<sub>X</sub> activity. The candidate or test compounds or agents that may bind to a FCTR<sub>X</sub> polypeptide may have a molecular weight around 50 Da, 100 Da, 150 Da, 300 Da, 330 Da, 350 Da, 400 Da, 500 Da, 750 Da, 1000 Da, 1250 Da, 1500 Da, 1750 Da, 2000 Da, 5000 Da, 10,000 Da, 25,000 Da, 50,000 Da, 75,000 Da, 100,000 Da or more than 100,000 Da. In certain embodiments, the candidate substance that binds to a FCTR<sub>X</sub> polypeptide has a molecular weight not more than about 1500 Da.

Details of functional assays are provided herein further below. Any of the assays described, as well as additional assays known to practitioners in the fields of pharmacology, hematology, internal medicine, oncology and the like, may be employed in order to screen candidate substance for their properties as therapeutic agents. As noted, the therapeutic agents of the invention encompass proteins, polypeptides, nucleic acids or polynucleotides, peptides, peptidomimetics, small molecules including agonists or antagonists, or other drugs described herein.

In one embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of a FCTR<sub>X</sub> protein or polypeptide or biologically active portion thereof. The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the "one-bead one-compound" library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam (1997) *Anticancer Drug Des* 12:145).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc Natl Acad Sci U.S.A.* 90:6909; Erb *et al.* (1994) *Proc Natl Acad Sci U.S.A.* 91:11422; Zuckermann *et al.* (1994) *J Med Chem* 37:2678; Cho *et*

*al.* (1993) *Science* 261:1303; Carrell *et al.* (1994) *Angew Chem Int Ed Engl* 33:2059; Carrell *et al.* (1994) *Angew Chem Int Ed Engl* 33:2061; and Gallop *et al.* (1994) *J Med Chem* 37:1233.

Libraries of compounds may be presented in solution (*e.g.*, Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), on chips (Fodor (1993) *Nature* 364:555-556), bacteria (Ladner U.S. Pat. No. 5,223,409), spores (Ladner USP '409), plasmids (Cull *et al.* (1992) *Proc Natl Acad Sci USA* 89:1865-1869) or on phage (Scott and Smith (1990) *Science* 249:386-390; Devlin (1990) *Science* 249:404-406; Cwirla *et al.* (1990) *Proc Natl Acad Sci U.S.A.* 87:6378-6382; Felici (1991) *J Mol Biol* 222:301-310; Ladner above.).

In one embodiment, an assay is a cell-based assay in which a cell which expresses a membrane-bound form of a FCTR<sub>X</sub> protein, or a biologically active portion thereof, on the cell surface is contacted with a test compound and the ability of the test compound to bind to a FCTR<sub>X</sub> protein determined. The cell, for example, can be of mammalian origin or a yeast cell. Determining the ability of the test compound to bind to the FCTR<sub>X</sub> protein can be accomplished, for example, by coupling the test compound with a radioisotope or enzymatic label such that binding of the test compound to the FCTR<sub>X</sub> protein or biologically active portion thereof can be determined by detecting the labeled compound in a complex. For example, test compounds can be labeled with <sup>125</sup>I, <sup>35</sup>S, <sup>14</sup>C, or <sup>3</sup>H, either directly or indirectly, and the radioisotope detected by direct counting of radioemission or by scintillation counting. Alternatively, test compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product. In one embodiment, the assay comprises contacting a cell which expresses a membrane-bound form of a FCTR<sub>X</sub> protein, or a biologically active portion thereof, on the cell surface with a known compound which binds a FCTR<sub>X</sub> to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a FCTR<sub>X</sub> protein, wherein determining the ability of the test compound to interact with a FCTR<sub>X</sub> protein comprises determining the ability of the test compound to preferentially bind to a FCTR<sub>X</sub> or a biologically active portion thereof as compared to the known compound.

In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a membrane-bound form of a FCTR<sub>X</sub> protein, or a biologically active portion thereof, on the cell surface with a test compound and determining the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the FCTR<sub>X</sub> protein or biologically active portion thereof. Determining the ability of the test compound to modulate

the activity of a FCTR<sub>X</sub> polypeptide or a biologically active portion thereof can be accomplished, for example, by determining the ability of the FCTR<sub>X</sub> protein to bind to or interact with a FCTR<sub>X</sub> target molecule. As used herein, a "target molecule" is a molecule with which a FCTR<sub>X</sub> protein binds or interacts in nature, for example, a molecule on the surface of a cell which expresses a FCTR<sub>X</sub> interacting protein, a molecule on the surface of a second cell, a molecule in the extracellular milieu, a molecule associated with the internal surface of a cell membrane or a cytoplasmic molecule. A FCTR<sub>X</sub> target molecule can be a non-FCTR<sub>X</sub> molecule or a FCTR<sub>X</sub> protein or polypeptide of the present invention. In one embodiment, a FCTR<sub>X</sub> target molecule is a component of a signal transduction pathway that facilitates transduction of an extracellular signal (*e.g.*, a signal generated by binding of a compound to a membrane-bound FCTR<sub>X</sub> molecule) through the cell membrane and into the cell. The target, for example, can be a second intercellular protein that has catalytic activity or a protein that facilitates the association of downstream signaling molecules with the FCTR<sub>X</sub> polypeptide.

Determining the ability of the FCTR<sub>X</sub> protein to bind to or interact with a FCTR<sub>X</sub> target molecule can be accomplished by one of the methods described above for determining direct binding. In one embodiment, determining the ability of the FCTR<sub>X</sub> protein to bind to or interact with a FCTR<sub>X</sub> target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (*i.e.* intracellular  $\text{Ca}^{2+}$ , diacylglycerol,  $\text{IP}_3$ , etc.), detecting catalytic/enzymatic activity of the target an appropriate substrate, detecting the induction of a reporter gene (comprising a FCTR<sub>X</sub>-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, *e.g.*, luciferase), or detecting a cellular response, for example, cell survival, cellular differentiation, or cell proliferation.

In yet another embodiment, an assay of the present invention is a cell-free assay comprising contacting a FCTR<sub>X</sub> protein or biologically active portion thereof with a test compound and determining the ability of the test compound to bind to the FCTR<sub>X</sub> protein or biologically active portion thereof. Binding of the test compound to the FCTR<sub>X</sub> protein can be determined either directly or indirectly as described above. In one embodiment, the assay comprises contacting the FCTR<sub>X</sub> protein or biologically active portion thereof with a known compound which binds FCTR<sub>X</sub> to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a FCTR<sub>X</sub> protein, wherein determining the ability of the test compound to interact with a FCTR<sub>X</sub>

protein comprises determining the ability of the test compound to preferentially bind to a FCTR<sub>X</sub> or biologically active portion thereof as compared to the known compound.

In another embodiment, an assay is a cell-free assay comprising contacting a FCTR<sub>X</sub> protein or biologically active portion thereof with a test compound and determining the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the FCTR<sub>X</sub> protein or biologically active portion thereof. Determining the ability of the test compound to modulate the activity of a FCTR<sub>X</sub> polypeptide can be accomplished, for example, by determining the ability of the FCTR<sub>X</sub> protein to bind to a FCTR<sub>X</sub> target molecule by one of the methods described above for determining direct binding. In an alternative embodiment, determining the ability of the test compound to modulate the activity of a FCTR<sub>X</sub> polypeptide can be accomplished by determining the ability of the FCTR<sub>X</sub> protein further modulate a FCTR<sub>X</sub> target molecule. For example, the catalytic/enzymatic activity of the target molecule on an appropriate substrate can be determined as previously described.

In yet another embodiment, the cell-free assay comprises contacting the FCTR<sub>X</sub> protein or biologically active portion thereof with a known compound which binds a FCTR<sub>X</sub> polypeptide to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a FCTR<sub>X</sub> protein, wherein determining the ability of the test compound to interact with a FCTR<sub>X</sub> protein comprises determining the ability of the FCTR<sub>X</sub> protein to preferentially bind to or modulate the activity of a FCTR<sub>X</sub> target molecule.

The cell-free assays of the present invention are amenable to use of both a soluble form or a membrane-bound form of a FCTR<sub>X</sub> polypeptide. In the case of cell-free assays comprising the membrane-bound form of a FCTR<sub>X</sub> polypeptide, it may be desirable to utilize a solubilizing agent such that the membrane-bound form of a FCTR<sub>X</sub> polypeptide is maintained in solution. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton<sup>®</sup> X-100, Triton<sup>®</sup> X-114, Thesit<sup>®</sup>, Isotridecypoly(ethylene glycol ether)<sub>n</sub>, N-dodecyl--N,N-dimethyl-3-ammonio-1-propane sulfonate, 3-(3-cholamidopropyl)dimethylamminiol-1-propane sulfonate (CHAPS), or 3-(3-cholamidopropyl)dimethylamminiol-2-hydroxy-1-propane sulfonate (CHAPSO).

It may be desirable to immobilize either a FCTR<sub>X</sub> polypeptide or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to a FCTR<sub>X</sub> polypeptide, or interaction of a FCTR<sub>X</sub> polypeptide with a target molecule in the presence and



absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided that adds a domain that allows one or both of the proteins to be bound to a matrix. For example, GST-FCTR<sub>X</sub> polypeptide fusion proteins or GST-target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtiter plates, that are then combined with the test compound or the test compound and either the non-adsorbed target protein or a FCTR<sub>X</sub> protein, and the mixture is incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of a FCTR<sub>X</sub> binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either the FCTR<sub>X</sub> polypeptide or its target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated FCTR<sub>X</sub> protein or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with FCTR<sub>X</sub> protein or target molecules, but which do not interfere with binding of the FCTR<sub>X</sub> protein to its target molecule, can be derivatized to the wells of the plate, and unbound target or FCTR<sub>X</sub> protein trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the FCTR<sub>X</sub> protein or target molecule, as well as enzyme-linked assays that rely on detecting an enzymatic activity associated with the FCTR<sub>X</sub> protein or target molecule.

In another embodiment, modulators of a FCTR<sub>X</sub> expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of a FCTR<sub>X</sub> mRNA or protein in the cell is determined. The level of expression of a FCTR<sub>X</sub> mRNA or protein in the presence of the candidate compound is compared to the level of expression of a FCTR<sub>X</sub> mRNA or protein in the absence of the candidate compound. The candidate compound can then be identified as a modulator of a FCTR<sub>X</sub> expression based on this comparison. For

example, when expression of a FCTR<sub>X</sub> mRNA or protein is greater (statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of a FCTR<sub>X</sub> mRNA or protein expression.

Alternatively, when expression of a FCTR<sub>X</sub> mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of a FCTR<sub>X</sub> mRNA or protein expression. The level of a FCTR<sub>X</sub> mRNA or protein expression in the cells can be determined by methods described herein for detecting FCTR<sub>X</sub> mRNA or protein.

In yet another aspect of the invention, the FCTR<sub>X</sub> proteins can be used as "bait proteins" in a two-hybrid assay or three hybrid assay (see, *e.g.*, U.S. Pat. No. 5,283,317; Zervos *et al.* (1993) *Cell* 72:223-232; Madura *et al.* (1993) *J Biol Chem* 268:12046-12054; Bartel *et al.* (1993) *Biotechniques* 14:920-924; Iwabuchi *et al.* (1993) *Oncogene* 8:1693-1696; and Brent WO94/10300), to identify other proteins that bind to or interact with the FCTR<sub>X</sub> ("FCTR<sub>X</sub>-binding proteins" or "FCTR<sub>X</sub>-bp") and modulate FCTR<sub>X</sub> activity. Such FCTR<sub>X</sub>-binding proteins are also likely to be involved in the propagation of signals by the FCTR<sub>X</sub> proteins as, for example, upstream or downstream elements of the FCTR<sub>X</sub> pathway.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for a FCTR<sub>X</sub> is fused to a gene encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, *in vivo*, forming a FCTR<sub>X</sub>-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ) that is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene that encodes the protein which interacts with the FCTR<sub>X</sub>.

Screening can also be performed *in vivo*. For example, in one embodiment, the invention includes a method for screening for a modulator of activity or of latency or predisposition to a FCTR<sub>X</sub>-associated disorder by administering a test compound or to a test animal at increased risk for a FCTR<sub>X</sub>-associated disorder. In some embodiments, the test animal recombinantly expresses a FCTR<sub>X</sub> polypeptide. Activity of the polypeptide in the test

animal after administering the compound is measured, and the activity of the protein in the test animal is compared to the activity of the polypeptide in a control animal not administered said polypeptide. A change in the activity of said polypeptide in said test animal relative to the control animal indicates the test compound is a modulator of latency of or predisposition to a FCTRX-associated disorder.

In some embodiments, the test animal is a recombinant test animal that expresses a test protein transgene or expresses the transgene under the control of a promoter at an increased level relative to a wild-type test animal. Preferably, the promoter is not the native gene promoter of the transgene.

This invention further pertains to novel agents identified by the above-described screening assays and uses thereof for treatments as described herein.

### **Detection Assays**

Portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. For example, these sequences can be used to: (i) map their respective genes on a chromosome; and, thus, locate gene regions associated with genetic disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample.

The FCTRX sequences of the present invention can also be used to identify individuals from minute biological samples. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identification. The sequences of the present invention are useful as additional DNA markers for RFLP ("restriction fragment length polymorphisms," described in U.S. Pat. No. 5,272,057).

Furthermore, the sequences of the present invention can be used to provide an alternative technique that determines the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the FCTRX sequences described herein can be used to prepare two PCR primers from the 5' and 3' ends of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it.

Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The sequences of the present invention can be used to obtain such identification sequences from individuals and from tissue. The FCTRX sequences of the invention uniquely represent portions of the human genome. Allelic variation

occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. It is estimated that allelic variation between individual humans occurs with a frequency of about once per each 500 bases. Much of the allelic variation is due to single nucleotide polymorphisms (SNPs), which include restriction fragment length polymorphisms (RFLPs).

Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. The noncoding sequences of SEQ ID NOS:1, 3, 5, 7, 9 and 11, as described above, can comfortably provide positive individual identification with a panel of perhaps 10 to 1,000 primers that each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences are used, a more appropriate number of primers for positive individual identification would be 500-2,000.

#### **Use Of Partial FCTR X Sequences In Forensic Biology**

DNA-based identification techniques based on FCTR X nucleic acid sequences or polypeptide sequences can also be used in forensic biology. Forensic biology is a scientific field employing genetic typing of biological evidence found at a crime scene as a means for positively identifying, for example, a perpetrator of a crime. To make such an identification, PCR technology can be used to amplify DNA sequences taken from very small biological samples such as tissues, *e.g.*, hair or skin, or body fluids, *e.g.*, blood, saliva, or semen found at a crime scene. The amplified sequence can then be compared to a standard, thereby allowing identification of the origin of the biological sample.

The sequences of the present invention can be used to provide polynucleotide reagents, *e.g.*, PCR primers, targeted to specific loci in the human genome, that can enhance the reliability of DNA-based forensic identifications by, for example, providing another "identification marker" (*i.e.* another DNA sequence that is unique to a particular individual). As mentioned above, actual base sequence information can be used for identification as an accurate alternative to patterns formed by restriction enzyme generated fragments. Sequences targeted to noncoding regions of SEQ ID NOS:1, 3, 5, 7, 9 and 11 are particularly appropriate for this use as greater numbers of polymorphisms occur in the noncoding regions, making it easier to differentiate individuals using this technique. Examples of polynucleotide reagents include the FCTR X sequences or portions thereof, *e.g.*, fragments derived from the noncoding regions of one or more of SEQ ID NOS:1, 3, 5, 7, 9 and 11, having a length of at least 20 bases, preferably at least 30 bases.

The FCTR<sub>X</sub> sequences described herein can further be used to provide polynucleotide reagents, *e.g.*, labeled or label-able probes that can be used, for example, in an *in situ* hybridization technique, to identify a specific tissue, *e.g.*, brain tissue, etc. This can be very useful in cases where a forensic pathologist is presented with a tissue of unknown origin. Panels of such FCTR<sub>X</sub> probes can be used to identify tissue by species and/or by organ type.

In a similar fashion, these reagents, *e.g.*, FCTR<sub>X</sub> primers or probes can be used to screen tissue culture for contamination (*i.e.* screen for the presence of a mixture of different types of cells in a culture).

### **Predictive Medicine**

The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, pharmacogenomics, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the present invention relates to diagnostic assays for determining a FCTR<sub>X</sub> protein and/or nucleic acid expression as well as FCTR<sub>X</sub> activity, in the context of a biological sample (*e.g.*, blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant FCTR<sub>X</sub> expression or activity. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with a FCTR<sub>X</sub> protein, nucleic acid expression or activity. For example, mutations in a FCTR<sub>X</sub> gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of a disorder characterized by or associated with FCTR<sub>X</sub> protein, nucleic acid expression or activity.

Another aspect of the invention provides methods for determining FCTR<sub>X</sub> protein, nucleic acid expression or FCTR<sub>X</sub> activity in an individual to thereby select appropriate therapeutic or prophylactic agents for that individual (referred to herein as "pharmacogenomics"). Pharmacogenomics allows for the selection of agents (*e.g.*, drugs) for therapeutic or prophylactic treatment of an individual based on the genotype of the individual (*e.g.*, the genotype of the individual examined to determine the ability of the individual to respond to a particular agent.)

Yet another aspect of the invention pertains to monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of a FCTR<sub>X</sub> in clinical trials.

These and other agents are described in further detail in the following sections.

**Diagnostic Assays**

Other conditions in which proliferation of cells plays a role include tumors, restenosis, psoriasis, Dupuytren's contracture, diabetic complications, Kaposi's sarcoma and rheumatoid arthritis.

A FCTR<sub>X</sub> polypeptide may be used to identify an interacting polypeptide a sample or tissue. The method comprises contacting the sample or tissue with the FCTR<sub>X</sub>, allowing formation of a complex between the FCTR<sub>X</sub> polypeptide and the interacting polypeptide, and detecting the complex, if present.

The proteins of the invention may be used to stimulate production of antibodies specifically binding the proteins. Such antibodies may be used in immunodiagnostic procedures to detect the occurrence of the protein in a sample. The proteins of the invention may be used to stimulate cell growth and cell proliferation in conditions in which such growth would be favorable. An example would be to counteract toxic side effects of chemotherapeutic agents on, for example, hematopoiesis and platelet formation, linings of the gastrointestinal tract, and hair follicles. They may also be used to stimulate new cell growth in neurological disorders including, for example, Alzheimer's disease. Alternatively, antagonistic treatments may be administered in which an antibody specifically binding the FCTR<sub>X</sub>-like proteins of the invention would abrogate the specific growth-inducing effects of the proteins. Such antibodies may be useful, for example, in the treatment of proliferative disorders including various tumors and benign hyperplasias.

Polynucleotides or oligonucleotides corresponding to any one portion of the FCTR<sub>X</sub> nucleic acids of SEQ ID NOS:1, 3, 5, 7, 9 and 11 may be used to detect DNA containing a corresponding ORF gene, or detect the expression of a corresponding FCTR<sub>X</sub> gene, or FCTR<sub>X</sub>-like gene. For example, a FCTR<sub>X</sub> nucleic acid expressed in a particular cell or tissue, as noted in Table 3, can be used to identify the presence of that particular cell type.

An exemplary method for detecting the presence or absence of a FCTR<sub>X</sub> polypeptide in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting a FCTR<sub>X</sub> protein or nucleic acid (*e.g.*, mRNA, genomic DNA) that encodes a FCTR<sub>X</sub> protein such that the presence of a FCTR<sub>X</sub> polypeptide is detected in the biological sample. An agent for detecting a FCTR<sub>X</sub> mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to a FCTR<sub>X</sub> mRNA or genomic DNA. The nucleic acid probe can be, for example, a full-length FCTR<sub>X</sub> nucleic acid, such as the nucleic acid of SEQ ID NOS:1, 3, 5, 7, 9 and 11, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or

500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to a FCTR<sub>X</sub> mRNA or genomic DNA, as described above. Other suitable probes for use in the diagnostic assays of the invention are described herein.

An agent for detecting a FCTR<sub>X</sub> protein is an antibody capable of binding to a FCTR<sub>X</sub> protein, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (*e.g.*, Fab or F(ab')<sub>2</sub>) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect a FCTR<sub>X</sub> mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of a FCTR<sub>X</sub> mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of a FCTR<sub>X</sub> protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. *In vitro* techniques for detection of a FCTR<sub>X</sub> genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of a FCTR<sub>X</sub> protein include introducing into a subject a labeled anti-FCTR<sub>X</sub> antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

In one embodiment, the biological sample contains protein molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject.

In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting a FCTR<sub>X</sub> protein, mRNA, or genomic DNA, such that the presence of a FCTR<sub>X</sub> protein, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of a FCTR<sub>X</sub> protein, mRNA or genomic DNA in the control sample with the presence of a FCTR<sub>X</sub> protein, mRNA or genomic DNA in the test sample.

The invention also encompasses kits for detecting the presence of a FCTR<sub>X</sub> polypeptide in a biological sample. For example, the kit can comprise: a labeled compound or agent capable of detecting a FCTR<sub>X</sub> protein or mRNA in a biological sample; means for determining the amount of a FCTR<sub>X</sub> polypeptide in the sample; and means for comparing the amount of a FCTR<sub>X</sub> polypeptide in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect a FCTR<sub>X</sub> protein or nucleic acid.

### **Prognostic Assays**

The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a disease or disorder associated with aberrant FCTR<sub>X</sub> polypeptide expression or activity. For example, the assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with a FCTR<sub>X</sub> protein, nucleic acid expression or activity in, *e.g.*, proliferative or differentiative disorders such as hyperplasias, tumors, restenosis, psoriasis, Dupuytren's contracture, diabetic complications, or rheumatoid arthritis, etc.; and glia-associated disorders such as cerebral lesions, diabetic neuropathies, cerebral edema, senile dementia, Alzheimer's disease, etc. Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for developing a disease or disorder. Thus, the present invention provides a method for identifying a disease or disorder associated with aberrant FCTR<sub>X</sub> expression or activity in which a test sample is obtained from a subject and a FCTR<sub>X</sub> protein or nucleic acid (*e.g.*, mRNA, genomic DNA) is detected, wherein the presence of a FCTR<sub>X</sub> protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant FCTR<sub>X</sub> expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (*e.g.*, serum), cell sample, or tissue.

Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant FCTR<sub>X</sub> expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for a disorder, such as a proliferative disorder, differentiative disorder, glia-associated disorders, etc. Thus, the present invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with aberrant FCTR<sub>X</sub> expression or activity in



which a test sample is obtained and a FCTR<sub>X</sub> protein or nucleic acid is detected (*e.g.*, wherein the presence of a FCTR<sub>X</sub> protein or nucleic acid is diagnostic for a subject that can be administered the agent to treat a disorder associated with aberrant FCTR<sub>X</sub> expression or activity.)

The methods of the invention can also be used to detect genetic lesions in a FCTR<sub>X</sub> gene, thereby determining if a subject with the lesioned gene is at risk for, or suffers from, a proliferative disorder, differentiative disorder, glia-associated disorder, etc. In various embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic lesion characterized by at least one of an alteration affecting the integrity of a gene encoding a FCTR<sub>X</sub> protein, or the mis-expression of the FCTR<sub>X</sub> gene. For example, such genetic lesions can be detected by ascertaining the existence of at least one of (1) a deletion of one or more nucleotides from a FCTR<sub>X</sub> gene; (2) an addition of one or more nucleotides to a FCTR<sub>X</sub> gene; (3) a substitution of one or more nucleotides of a FCTR<sub>X</sub> gene, (4) a chromosomal rearrangement of a FCTR<sub>X</sub> gene; (5) an alteration in the level of a messenger RNA transcript of a FCTR<sub>X</sub> gene, (6) aberrant modification of a FCTR<sub>X</sub> gene, such as of the methylation pattern of the genomic DNA, (7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of a FCTR<sub>X</sub> gene, (8) a non-wild type level of a protein, (9) allelic loss of a FCTR<sub>X</sub> gene, and (10) inappropriate post-translational modification of a FCTR<sub>X</sub> protein. As described herein, there are a large number of assay techniques known in the art which can be used for detecting lesions in a FCTR<sub>X</sub> gene. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject. However, any biological sample containing nucleated cells may be used, including, for example, buccal mucosal cells.

In certain embodiments, detection of the lesion involves the use of a probe/primer in a polymerase chain reaction (PCR) (see, *e.g.*, U.S. Pat. Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (see, *e.g.*, Landegran *et al.* (1988) *Science* 241:1077-1080; and Nakazawa *et al.* (1994) *PNAS* 91:360-364), the latter of which can be particularly useful for detecting point mutations in the FCTR<sub>X</sub> gene (see Abravaya *et al.* (1995) *Nucl Acids Res* 23:675-682). This method can include the steps of collecting a sample of cells from a patient, isolating nucleic acid (*e.g.*, genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers that specifically hybridize to a FCTR<sub>X</sub> gene under conditions such that hybridization and amplification of the FCTR<sub>X</sub> gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification

product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (Guatelli *et al.*, 1990, *Proc Natl Acad Sci USA* 87:1874-1878), transcriptional amplification system (Kwoh, *et al.*, 1989, *Proc Natl Acad Sci USA* 86:1173-1177), Q-Beta Replicase (Lizardi *et al.*, 1988, *BioTechnology* 6:1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in a FCTRX gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (see, for example, U.S. Pat. No. 5,493,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in a FCTRX nucleic acid of the invention can be identified by hybridizing a sample and control nucleic acids, *e.g.*, DNA or RNA, to high density arrays containing hundreds or thousands of oligonucleotides probes (Cronin *et al.* (1996) *Human Mutation* 7: 244-255; Kozal *et al.* (1996) *Nature Medicine* 2: 753-759). For example, genetic mutations in a FCTRX of the invention can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin *et al.* above. Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the FCTRX gene and detect mutations by comparing the sequence of the sample FCTRX gene with the corresponding wild-type (control) sequence.

Examples of sequencing reactions include those based on techniques developed by Maxim and Gilbert (1977) *PNAS* 74:560 or Sanger (1977) *PNAS* 74:5463. It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays (Naeve *et al.*, (1995) *Biotechniques* 19:448), including sequencing by mass spectrometry (see, *e.g.*, PCT International Publ. No. WO 94/16101; Cohen *et al.* (1996) *Adv Chromatogr* 36:127-162; and Griffin *et al.* (1993) *Appl Biochem Biotechnol* 38:147-159).

Other methods for detecting mutations in the FCTR<sub>X</sub> gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers *et al.* (1985) *Science* 230:1242). In general, the art technique of "mismatch cleavage" starts by providing heteroduplexes of formed by hybridizing (labeled) RNA or DNA containing the wild-type FCTR<sub>X</sub> sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent that cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with S1 nuclease to enzymatically digesting the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. See, for example, Cotton *et al* (1988) *Proc Natl Acad Sci USA* 85:4397; Saleeba *et al* (1992) *Methods Enzymol* 217:286-295. In an embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in FCTR<sub>X</sub> cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu *et al.* (1994) *Carcinogenesis* 15:1657-1662). According to an exemplary embodiment, a probe based on a FCTR<sub>X</sub> sequence, *e.g.*, a wild-type FCTR<sub>X</sub> sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. See, for example, U.S. Pat. No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in FCTR<sub>X</sub> genes. For example, single strand conformation polymorphism (SSCP)

may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita *et al.* (1989) *Proc Natl Acad Sci USA* 86:2766, see also Cotton (1993) *Mutat Res* 285:125-144; Hayashi (1992) *Genet Anal Tech Appl* 9:73-79). Single-stranded DNA fragments of sample and control a FCTR<sub>X</sub> nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA, rather than DNA, in which the secondary structure is more sensitive to a change in sequence. In one embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility. See, *e.g.*, Keen *et al.* (1991) *Trends Genet* 7:5.

In yet another embodiment the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE). See, *e.g.*, Myers *et al.* (1985) *Nature* 313:495. When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA. See, *e.g.*, Rosenbaum and Reissner (1987) *Biophys Chem* 265:12753.

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions that permit hybridization only if a perfect match is found. See, *e.g.*, Saiki *et al.* (1986) *Nature* 324:163; Saiki *et al.* (1989) *Proc Natl Acad. Sci USA* 86:6230. Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele specific amplification technology that depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs *et al.* (1989) *Nucleic Acids Res* 17:2437-2448) or at the extreme 3' end of one primer where, under

appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection. See, e.g., Gasparini *et al* (1992) *Mol Cell Probes* 6:1. It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification. See, e.g., Barany (1991) *Proc Natl Acad Sci USA* 88:189. In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence, making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, e.g., in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving a FCTR<sub>X</sub> gene.

Furthermore, any cell type or tissue, preferably peripheral blood leukocytes, in which a FCTR<sub>X</sub> of the invention is expressed may be utilized in the prognostic assays described herein. However, any biological sample containing nucleated cells may be used, including, for example, buccal mucosal cells.

### **Pharmacogenomics**

Agents, or modulators that have a stimulatory or inhibitory effect on FCTR<sub>X</sub> activity (e.g., FCTR<sub>X</sub> gene expression), as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) disorders (e.g., neurological, cancer-related or gestational disorders) associated with aberrant FCTR<sub>X</sub> activity. In conjunction with such treatment, the pharmacogenomics (i.e., the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) of the individual may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, the pharmacogenomics of the individual permits the selection of effective agents (e.g., drugs) for prophylactic or therapeutic treatments based on a consideration of the individual's genotype. Such pharmacogenomics can further be used to determine appropriate dosages and therapeutic regimens. Accordingly, the activity of a FCTR<sub>X</sub> protein, expression of a FCTR<sub>X</sub> nucleic acid, or mutation content of a FCTR<sub>X</sub> genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See *e.g.*, Eichelbaum, 1996, *Clin Exp Pharmacol Physiol*, 23:983-985 and Linder, 1997, *Clin Chem*, 43:254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare defects or as polymorphisms. For example, glucose-6-phosphate dehydrogenase (G6PD) deficiency is a common inherited enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (*e.g.*, N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. The other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

Thus, the activity of a FCTR<sub>X</sub> protein, expression of a FCTR<sub>X</sub> nucleic acid, or mutation content of a FCTR<sub>X</sub> genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual. In addition, pharmacogenetic studies can be used to apply genotyping of polymorphic alleles encoding drug-metabolizing enzymes to the identification of an individual's drug responsiveness phenotype. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when

treating a subject with a FCTR<sub>X</sub> modulator, such as a modulator identified by one of the exemplary screening assays described herein.

### Monitoring Clinical Efficacy

Monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of a FCTR<sub>X</sub> (*e.g.*, the ability to modulate aberrant cell proliferation and/or differentiation) can be applied in basic drug screening and in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase FCTR<sub>X</sub> gene expression, protein levels, or upregulate FCTR<sub>X</sub> activity, can be monitored in clinical trials of subjects exhibiting decreased FCTR<sub>X</sub> gene expression, protein levels, or downregulated FCTR<sub>X</sub> activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease FCTR<sub>X</sub> gene expression, protein levels, or downregulate FCTR<sub>X</sub> activity, can be monitored in clinical trials of subjects exhibiting increased FCTR<sub>X</sub> gene expression, protein levels, or upregulated FCTR<sub>X</sub> activity. In such clinical trials, the expression or activity of a FCTR<sub>X</sub> and, preferably, other genes that have been implicated in, for example, a proliferative or neurological disorder, can be used as a "read out" or marker of the responsiveness of a particular cell. Other FCTR<sub>X</sub>-associated disorders include, *e.g.*, cancers, cell proliferation disorders, anxiety disorders; CNS disorders; diabetes; obesity; and infectious disease.

For example, genes, including genes encoding a FCTR<sub>X</sub> of the invention, that are modulated in cells by treatment with an agent (*e.g.*, compound, drug or small molecule) that modulates a FCTR<sub>X</sub> activity (*e.g.*, identified in a screening assay as described herein) can be identified. Thus, to study the effect of agents on cellular proliferation disorders, for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of a FCTR<sub>X</sub> and other genes implicated in the disorder. The levels of gene expression (*i.e.*, a gene expression pattern) can be quantified by Northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein produced, by one of the methods as described herein, or by measuring the levels of activity of a gene or other genes. In this way, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during, treatment of the individual with the agent.

In one embodiment, the invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (*e.g.*, an agonist, antagonist, protein, peptide, nucleic acid, peptidomimetic, small molecule, or other drug candidate identified by the screening assays described herein) comprising the steps of (i) obtaining a pre-administration sample

from a subject prior to administration of the agent; (ii) detecting the level of expression of a FCTR protein, mRNA, or genomic DNA in the preadministration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the level of expression or activity of the FCTR protein, mRNA, or genomic DNA in the post-administration samples; (v) comparing the level of expression or activity of the FCTR protein, mRNA, or genomic DNA in the pre-administration sample with the FCTR protein, mRNA, or genomic DNA in the post administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of a FCTR to higher levels than detected, *i.e.*, to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of a FCTR to lower levels than detected, *i.e.*, to decrease the effectiveness of the agent.

### Methods of Treatment

The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant FCTR expression or activity.

Diseases and disorders that are characterized by increased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with Therapeutics that antagonize (*i.e.*, reduce or inhibit) activity. Therapeutics that antagonize activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be utilized include, but are not limited to, (i) a FCTR polypeptide, or analogs, derivatives, fragments or homologs thereof; (ii) antibodies to a FCTR peptide; (iii) nucleic acids encoding a FCTR peptide; (iv) administration of antisense nucleic acid and nucleic acids that are "dysfunctional" (*i.e.*, due to a heterologous insertion within the coding sequences of coding sequences to a FCTR polypeptide) that are utilized to "knockout" endogenous function of a FCTR polypeptide by homologous recombination (see, *e.g.*, Capecchi, 1989, *Science* 244: 1288-1292); or (v) modulators (*i.e.*, inhibitors, agonists and antagonists, including additional peptide mimetic of the invention or antibodies specific to a peptide of the invention) that alter the interaction between a FCTR peptide and its binding partner.

Diseases and disorders that are characterized by decreased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with Therapeutics that increase (*i.e.*, are agonists to) activity. Therapeutics that upregulate activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be



utilized include, but are not limited to, a polypeptide, a peptide, or analogs, derivatives, fragments or homologs thereof; or an agonist that increases bioavailability.

Increased or decreased levels can be readily detected by quantifying peptide and/or RNA, by obtaining a patient tissue sample (*e.g.*, from biopsy tissue) and assaying it *in vitro* for RNA or polypeptide levels, structure and/or activity of the expressed polypeptides (or mRNAs encoding a FCTR<sub>X</sub> polypeptide). Methods that are well-known within the art include, but are not limited to, immunoassays (*e.g.*, by Western blot analysis, immunoprecipitation followed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, immunocytochemistry, etc.) and/or hybridization assays to detect expression of mRNAs (*e.g.*, Northern assays, dot blots, *in situ* hybridization, etc.).

In one aspect, the invention provides a method for preventing, in a subject, a disease or condition associated with aberrant FCTR<sub>X</sub> expression or activity, by administering to the subject an agent that modulates FCTR<sub>X</sub> expression or at least one FCTR<sub>X</sub> activity. Subjects at risk for a disease that is caused or contributed to by aberrant FCTR<sub>X</sub> expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the FCTR<sub>X</sub> aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of a FCTR<sub>X</sub> aberrancy, for example, a FCTR<sub>X</sub> agonist or FCTR<sub>X</sub> antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein.

Another aspect of the invention pertains to methods of modulating FCTR<sub>X</sub> expression or activity for therapeutic purposes. The modulatory method of the invention involves contacting a cell with an agent that modulates one or more of the activities of a FCTR<sub>X</sub> protein activity associated with the cell. An agent that modulates a FCTR<sub>X</sub> protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring cognate ligand of a FCTR<sub>X</sub> protein, a peptide, a FCTR<sub>X</sub> peptidomimetic, or other small molecule. In one embodiment, the agent stimulates one or more a FCTR<sub>X</sub> protein activity. Examples of such stimulatory agents include active a FCTR<sub>X</sub> protein and a nucleic acid molecule encoding a FCTR<sub>X</sub> polypeptide that has been introduced into the cell. In another embodiment, the agent inhibits one or more a FCTR<sub>X</sub> protein activity. Examples of such inhibitory agents include antisense a FCTR<sub>X</sub> nucleic acid molecules and anti-FCTR<sub>X</sub> antibodies. These modulatory methods can be performed *in vitro* (*e.g.*, by culturing the cell with the agent) or, alternatively, *in vivo* (*e.g.*, by administering the agent to a subject). As

such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant expression or activity of a FCTR<sub>X</sub> protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (*e.g.*, an agent identified by a screening assay described herein), or combination of agents that modulates (*e.g.*, upregulates or downregulates) FCTR<sub>X</sub> expression or activity. In another embodiment, the method involves administering a FCTR<sub>X</sub> protein or nucleic acid molecule as therapy to compensate for reduced or aberrant FCTR<sub>X</sub> expression or activity.

### **Determination of the Biological Effect of a Therapeutic**

In various embodiments of the present invention, suitable *in vitro* or *in vivo* assays are utilized to determine the effect of a specific Therapeutic and whether its administration is indicated for treatment of the affected tissue.

In various specific embodiments, *in vitro* assays may be performed with representative cells of the type(s) involved in the patient's disorder, to determine if a given Therapeutic exerts the desired effect upon the cell type(s). Compounds for use in therapy may be tested in suitable animal model systems including, but not limited to rats, mice, chicken, cows, monkeys, rabbits, and the like, prior to testing in human subjects. Similarly, for *in vivo* testing, any of the animal model system known in the art may be used prior to administration to human subjects.

### **Malignancies**

Some FCTR<sub>X</sub> polypeptides are expressed in cancerous cells and are therefore implicated in the regulation of cell proliferation. Accordingly, Therapeutics of the present invention may be useful in the therapeutic or prophylactic treatment of diseases or disorders that are associated with cell hyperproliferation and/or loss of control of cell proliferation (*e.g.*, cancers, malignancies and tumors). For a review of such hyperproliferation disorders, see *e.g.*, Fishman, *et al.*, 1985. MEDICINE, 2nd ed., J.B. Lippincott Co., Philadelphia, PA.

Therapeutics of the present invention may be assayed by any method known within the art for efficacy in treating or preventing malignancies and related disorders. Such assays include, but are not limited to, *in vitro* assays utilizing transformed cells or cells derived from the patient's tumor, as well as *in vivo* assays using animal models of cancer or malignancies. Potentially effective Therapeutics are those that, for example, inhibit the proliferation of tumor-derived or transformed cells in culture or cause a regression of tumors in animal models, in comparison to the controls.

In the practice of the present invention, once a malignancy or cancer has been shown to be amenable to treatment by modulating (*i.e.*, inhibiting, antagonizing or agonizing) activity, that cancer or malignancy may subsequently be treated or prevented by the administration of a Therapeutic that serves to modulate protein function.

### **Premalignant Conditions**

The Therapeutics of the present invention, that are effective in the therapeutic or prophylactic treatment of cancer or malignancies may also be administered for the treatment of pre-malignant conditions and/or to prevent the progression of a pre-malignancy to a neoplastic or malignant state. Such prophylactic or therapeutic use is indicated in conditions known or suspected of preceding progression to neoplasia or cancer, in particular, where non-neoplastic cell growth consisting of hyperplasia, metaplasia or, most particularly, dysplasia has occurred. For a review of such abnormal cell growth see *e.g.*, Robbins & Angell, 1976. BASIC PATHOLOGY, 2nd ed., W.B. Saunders Co., Philadelphia, PA.

Hyperplasia is a form of controlled cell proliferation involving an increase in cell number in a tissue or organ, without significant alteration in its structure or function. For example, it has been demonstrated that endometrial hyperplasia often precedes endometrial cancer. Metaplasia is a form of controlled cell growth in which one type of mature or fully differentiated cell substitutes for another type of mature cell. Metaplasia may occur in epithelial or connective tissue cells. Dysplasia is generally considered a precursor of cancer, and is found mainly in the epithelia. Dysplasia is the most disorderly form of non-neoplastic cell growth, and involves a loss in individual cell uniformity and in the architectural orientation of cells. Dysplasia characteristically occurs where there exists chronic irritation or inflammation, and is often found in the cervix, respiratory passages, oral cavity, and gall bladder.

Alternatively, or in addition to the presence of abnormal cell growth characterized as hyperplasia, metaplasia, or dysplasia, the presence of one or more characteristics of a transformed or malignant phenotype displayed either *in vivo* or *in vitro* within a cell sample derived from a patient, is indicative of the desirability of prophylactic/therapeutic administration of a Therapeutic that possesses the ability to modulate activity of An aforementioned protein. Characteristics of a transformed phenotype include, but are not limited to: (i) morphological changes; (ii) looser substratum attachment; (iii) loss of cell-to-cell contact inhibition; (iv) loss of anchorage dependence; (v) protease release; (vi) increased sugar transport; (vii) decreased serum requirement; (viii) expression of fetal antigens, (ix)

disappearance of the 250 kDa cell-surface protein, and the like. See *e.g.*, Richards, *et al.*, 1986. MOLECULAR PATHOLOGY, W.B. Saunders Co., Philadelphia, PA.

In a specific embodiment of the present invention, a patient that exhibits one or more of the following predisposing factors for malignancy is treated by administration of an effective amount of a Therapeutic: (i) a chromosomal translocation associated with a malignancy (*e.g.*, the Philadelphia chromosome (*bcr/abl*) for chronic myelogenous leukemia and t(14;20) for follicular lymphoma, etc.); (ii) familial polyposis or Gardner's syndrome (possible forerunners of colon cancer); (iii) monoclonal gammopathy of undetermined significance (a possible precursor of multiple myeloma) and (iv) a first degree kinship with persons having a cancer or pre-cancerous disease showing a Mendelian (genetic) inheritance pattern (*e.g.*, familial polyposis of the colon, Gardner's syndrome, hereditary exostosis, polyendocrine adenomatosis, Peutz-Jeghers syndrome, neurofibromatosis of Von Recklinghausen, medullary thyroid carcinoma with amyloid production and pheochromocytoma, retinoblastoma, carotid body tumor, cutaneous melanocarcinoma, intraocular melanocarcinoma, xeroderma pigmentosum, ataxia telangiectasia, Chediak-Higashi syndrome, albinism, Fanconi's aplastic anemia and Bloom's syndrome).

In another embodiment, a Therapeutic of the present invention is administered to a human patient to prevent the progression to breast, colon, lung, pancreatic, or uterine cancer, or melanoma or sarcoma.

### **Hyperproliferative And Dysproliferative Disorders**

In one embodiment of the present invention, a Therapeutic is administered in the therapeutic or prophylactic treatment of hyperproliferative or benign dysproliferative disorders. The efficacy in treating or preventing hyperproliferative diseases or disorders of a Therapeutic of the present invention may be assayed by any method known within the art. Such assays include *in vitro* cell proliferation assays, *in vitro* or *in vivo* assays using animal models of hyperproliferative diseases or disorders, or the like. Potentially effective Therapeutics may, for example, promote cell proliferation in culture or cause growth or cell proliferation in animal models in comparison to controls.

Specific embodiments of the present invention are directed to the treatment or prevention of cirrhosis of the liver (a condition in which scarring has overtaken normal liver regeneration processes); treatment of keloid (hypertrophic scar) formation causing disfiguring of the skin in which the scarring process interferes with normal renewal; psoriasis (a common skin condition characterized by excessive proliferation of the skin and delay in proper cell fate

determination); benign tumors; fibrocystic conditions and tissue hypertrophy (e.g., benign prostatic hypertrophy).

### **Neurodegenerative Disorders**

Some FCTR proteins are found in cell types have been implicated in the deregulation of cellular maturation and apoptosis, which are both characteristic of neurodegenerative disease. Accordingly, Therapeutics of the invention, particularly but not limited to those that modulate (or supply) activity of an aforementioned protein, may be effective in treating or preventing neurodegenerative disease. Therapeutics of the present invention that modulate the activity of an aforementioned protein involved in neurodegenerative disorders can be assayed by any method known in the art for efficacy in treating or preventing such neurodegenerative diseases and disorders. Such assays include *in vitro* assays for regulated cell maturation or inhibition of apoptosis or *in vivo* assays using animal models of neurodegenerative diseases or disorders, or any of the assays described below. Potentially effective Therapeutics, for example but not by way of limitation, promote regulated cell maturation and prevent cell apoptosis in culture, or reduce neurodegeneration in animal models in comparison to controls.

Once a neurodegenerative disease or disorder has been shown to be amenable to treatment by modulation activity, that neurodegenerative disease or disorder can be treated or prevented by administration of a Therapeutic that modulates activity. Such diseases include all degenerative disorders involved with aging, especially osteoarthritis and neurodegenerative disorders.

### **Disorders Related To Organ Transplantation**

Some FCTR proteins can be associated with disorders related to organ transplantation, in particular but not limited to organ rejection. Therapeutics of the invention, particularly those that modulate (or supply) activity, may be effective in treating or preventing diseases or disorders related to organ transplantation. Therapeutics of the invention (particularly Therapeutics that modulate the levels or activity of an aforementioned protein) can be assayed by any method known in the art for efficacy in treating or preventing such diseases and disorders related to organ transplantation. Such assays include *in vitro* assays for using cell culture models as described below, or *in vivo* assays using animal models of diseases and disorders related to organ transplantation, see e.g., below. Potentially effective Therapeutics, for example but not by way of limitation, reduce immune rejection responses in animal models in comparison to controls.

Accordingly, once diseases and disorders related to organ transplantation are shown to be amenable to treatment by modulation of activity, such diseases or disorders can be treated or prevented by administration of a Therapeutic that modulates activity.

### **Cytokine and Cell Proliferation/Differentiation Activity**

A FCTR<sub>X</sub> protein or a cognate Therapeutic of the present invention may exhibit cytokine, cell proliferation (either inducing or inhibiting) or cell differentiation (either inducing or inhibiting) activity or may induce production of other cytokines in certain cell populations. Many protein factors discovered to date, including all known cytokines, have exhibited activity in one or more factor dependent cell proliferation assays, and hence the assays serve as a convenient confirmation of cytokine activity. The activity of a protein of the present invention is evidenced by any one of a number of routine factor dependent cell proliferation assays for cell lines including, without limitation, 32D, DA2, DA1G, T10, B9, B9/11, BaF3, MC9/G, M+ (preB M+), 2E8, RB5, DA1, 123, T1165, HT2, CTLL2, TF-1, Mo7e and CMK.

The activity of a protein of the invention may, among other means, be measured by the following methods: Assays for T-cell or thymocyte proliferation include without limitation those described in: CURRENT PROTOCOLS IN IMMUNOLOGY, Ed by Coligan *et al.*, Greene Publishing Associates and Wiley-Interscience (Chapter 3 and Chapter 7); Takai *et al.*, *J Immunol* 137:3494-3500, 1986; Bertagnoli *et al.*, *J Immunol* 145:1706-1712, 1990; Bertagnoli *et al.*, *Cell Immunol* 133:327-341, 1991; Bertagnoli, *et al.*, *J Immunol* 149:3778-3783, 1992; Bowman *et al.*, *J Immunol* 152:1756-1761, 1994.

Assays for cytokine production and/or proliferation of spleen cells, lymph node cells or thymocytes include, without limitation, those described by Kruisbeek and Shevach, In: CURRENT PROTOCOLS IN IMMUNOLOGY. Coligan *et al.*, eds. Vol 1, pp. 3.12.1-14, John Wiley and Sons, Toronto 1994; and by Schreiber, In: CURRENT PROTOCOLS IN IMMUNOLOGY. Coligan eds. Vol 1 pp. 6.8.1-8, John Wiley and Sons, Toronto 1994.

Assays for proliferation and differentiation of hematopoietic and lymphopoietic cells include, without limitation, those described by Bottomly *et al.*, In: CURRENT PROTOCOLS IN IMMUNOLOGY. Coligan *et al.*, eds. Vol 1 pp. 6.3.1-6.3.12, John Wiley and Sons, Toronto 1991; deVries *et al.*, *J Exp Med* 173:1205-1211, 1991; Moreau *et al.*, *Nature* 336:690-692, 1988; Greenberger *et al.*, *Proc Natl Acad Sci U.S.A.* 80:2931-2938, 1983; Nordan, In: CURRENT PROTOCOLS IN IMMUNOLOGY. Coligan *et al.*, eds. Vol 1 pp. 6.6.1-5, John Wiley and Sons, Toronto 1991; Smith *et al.*, *Proc Natl Acad Sci U.S.A.* 83:1857-1861, 1986; Measurement of human Interleukin 11-Bennett, *et al.* In: CURRENT PROTOCOLS IN IMMUNOLOGY. Coligan *et al.*,

eds. Vol 1 pp. 6.15.1 John Wiley and Sons, Toronto 1991; Ciarletta, *et al.*, In: CURRENT PROTOCOLS IN IMMUNOLOGY. Coligan *et al.*, eds. Vol 1 pp. 6.13.1, John Wiley and Sons, Toronto 1991.

Assays for T-cell clone responses to antigens (which will identify, among others, proteins that affect APC-T cell interactions as well as direct T-cell effects by measuring proliferation and cytokine production) include, without limitation, those described In: CURRENT PROTOCOLS IN IMMUNOLOGY. Coligan *et al.*, eds., Greene Publishing Associates and Wiley-Interscience (Chapter 3Chapter 6, Chapter 7); Weinberger *et al.*, *Proc Natl Acad Sci USA* 77:6091-6095, 1980; Weinberger *et al.*, *Eur J Immun* 11:405-411, 1981; Takai *et al.*, *J Immunol* 137:3494-3500, 1986; Takai *et al.*, *J Immunol* 140:508-512, 1988.

### **Immune Stimulating or Suppressing Activity**

A FCTR protein or a cognate Therapeutic of the present invention may also exhibit immune stimulating or immune suppressing activity, including without limitation the activities for which assays are described herein. A protein may be useful in the treatment of various immune deficiencies and disorders (including severe combined immunodeficiency (SCID)), *e.g.*, in regulating (up or down) growth and proliferation of T and/or B lymphocytes, as well as effecting the cytolytic activity of NK cells and other cell populations. These immune deficiencies may be genetic or be caused by vital (*e.g.*, HIV) as well as bacterial or fungal infections, or may result from autoimmune disorders. More specifically, infectious diseases caused by vital, bacterial, fungal or other infection may be treatable using a protein of the present invention, including infections by HIV, hepatitis viruses, herpes viruses, mycobacteria, *Leishmania* species., malaria species. and various fungal infections such as candidiasis. Of course, in this regard, a protein of the present invention may also be useful where a boost to the immune system generally may be desirable, *i.e.*, in the treatment of cancer.

Autoimmune disorders which may be treated using a protein or a cognate Therapeutic of the present invention include, for example, connective tissue disease, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes mellitus, myasthenia gravis, graft-versus-host disease and autoimmune inflammatory eye disease. Such a protein of the present invention may also be useful in the treatment of allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems. Other conditions, in which immune suppression is desired (including, for example, organ transplantation), may also be treatable using a protein of the present invention.

Using a protein or a cognate Therapeutic of the invention it may also be possible to modulate immune responses, in a number of ways. Down regulation may be in the form of inhibiting or blocking an immune response already in progress or may involve preventing the induction of an immune response. The functions of activated T cells may be inhibited by suppressing T cell responses or by inducing specific tolerance in T cells, or both.

Immunosuppression of T cell responses is generally an active, non-antigen-specific, process which requires continuous exposure of the T cells to the suppressive agent. Tolerance, which involves inducing non-responsiveness or energy in T cells, is distinguishable from immunosuppression in that it is generally antigen-specific and persists after exposure to the tolerizing agent has ceased. Operationally, tolerance can be demonstrated by the lack of a T cell response upon re-exposure to specific antigen in the absence of the tolerizing agent.

Down regulating or preventing one or more antigen functions (including without limitation B lymphocyte antigen functions (such as, for example, B7)), *e.g.*, preventing high level lymphokine synthesis by activated T cells, will be useful in situations of tissue, skin and organ transplantation and in graft-versus-host disease (GVHD). For example, blockage of T cell function should result in reduced tissue destruction in tissue transplantation. Typically, in tissue transplants, rejection of the transplant is initiated through its recognition as foreign by T cells, followed by an immune reaction that destroys the transplant. The administration of a molecule which inhibits or blocks interaction of a B7 lymphocyte antigen with its natural ligand(s) on immune cells (such as a soluble, monomeric form of a peptide having B7-2 activity alone or in conjunction with a monomeric form of a peptide having an activity of another B lymphocyte antigen (*e.g.*, B7-1, B7-3) or blocking antibody), prior to transplantation can lead to the binding of the molecule to the natural ligand(s) on the immune cells without transmitting the corresponding costimulatory signal. Blocking B lymphocyte antigen function in this matter prevents cytokine synthesis by immune cells, such as T cells, and thus acts as an immunosuppressant. Moreover, the lack of costimulation may also be sufficient to energize the T cells, thereby inducing tolerance in a subject. Induction of long-term tolerance by B lymphocyte antigen-blocking reagents may avoid the necessity of repeated administration of these blocking reagents. To achieve sufficient immunosuppression or tolerance in a subject, it may also be necessary to block the function of B lymphocyte antigens.

The efficacy of particular blocking reagents in preventing organ transplant rejection or GVHD can be assessed using animal models that are predictive of efficacy in humans. Examples of appropriate systems which can be used include allogeneic cardiac grafts in rats and xenogeneic pancreatic islet cell grafts in mice, both of which have been used to examine



the immunosuppressive effects of CTLA4Ig fusion proteins *in vivo* as described in Lenschow *et al.*, Science 257:789-792 (1992) and Turka *et al.*, Proc Natl Acad Sci USA, 89:11102-11105 (1992). In addition, murine models of GVHD (see Paul ed., FUNDAMENTAL IMMUNOLOGY, Raven Press, New York, 1989, pp. 846-847) can be used to determine the effect of blocking B lymphocyte antigen function *in vivo* on the development of that disease.

Blocking antigen function may also be therapeutically useful for treating autoimmune diseases. Many autoimmune disorders are the result of inappropriate activation of T cells that are reactive against self tissue and which promote the production of cytokines and auto-antibodies involved in the pathology of the diseases. Preventing the activation of autoreactive T cells may reduce or eliminate disease symptoms. Administration of reagents which block costimulation of T cells by disrupting receptor:ligand interactions of B lymphocyte antigens can be used to inhibit T cell activation and prevent production of auto-antibodies or T cell-derived cytokines which may be involved in the disease process. Additionally, blocking reagents may induce antigen-specific tolerance of autoreactive T cells which could lead to long-term relief from the disease. The efficacy of blocking reagents in preventing or alleviating autoimmune disorders can be determined using a number of well-characterized animal models of human autoimmune diseases. Examples include murine experimental autoimmune encephalitis, systemic lupus erythematosus in MRL/lpr/lpr mice or NZB hybrid mice, murine autoimmune collagen arthritis, diabetes mellitus in NOD mice and BB rats, and murine experimental myasthenia gravis (see Paul ed., FUNDAMENTAL IMMUNOLOGY, Raven Press, New York, 1989, pp. 840-856).

Upregulation of an antigen function (preferably a B lymphocyte antigen function), as a means of up regulating immune responses, may also be useful in therapy. Upregulation of immune responses may be in the form of enhancing an existing immune response or eliciting an initial immune response. For example, enhancing an immune response through stimulating B lymphocyte antigen function may be useful in cases of viral infection. In addition, systemic viral diseases such as influenza, the common cold, and encephalitis might be alleviated by the administration of stimulatory forms of B lymphocyte antigens systemically.

Alternatively, anti-viral immune responses may be enhanced in an infected patient by removing T cells from the patient, costimulating the T cells *in vitro* with viral antigen-pulsed APCs either expressing a peptide of the present invention or together with a stimulatory form of a soluble peptide of the present invention and reintroducing the *in vitro* activated T cells into the patient. Another method of enhancing anti-viral immune responses would be to isolate infected cells from a patient, transfect them with a nucleic acid encoding a protein of the

present invention as described herein such that the cells express all or a portion of the protein on their surface, and reintroduce the transfected cells into the patient. The infected cells would now be capable of delivering a costimulatory signal to, and thereby activate, T cells *in vivo*.

In another application, up regulation or enhancement of antigen function (preferably B lymphocyte antigen function) may be useful in the induction of tumor immunity. Tumor cells (*e.g.*, sarcoma, melanoma, lymphoma, leukemia, neuroblastoma, carcinoma) transfected with a nucleic acid encoding at least one peptide of the present invention can be administered to a subject to overcome tumor-specific tolerance in the subject. If desired, the tumor cell can be transfected to express a combination of peptides. For example, tumor cells obtained from a patient can be transfected *ex vivo* with an expression vector directing the expression of a peptide having B7-2-like activity alone, or in conjunction with a peptide having B7-1-like activity and/or B7-3-like activity. The transfected tumor cells are returned to the patient to result in expression of the peptides on the surface of the transfected cell. Alternatively, gene therapy techniques can be used to target a tumor cell for transfection *in vivo*.

The presence of the peptide of the present invention having the activity of a B lymphocyte antigen(s) on the surface of the tumor cell provides the necessary costimulation signal to T cells to induce a T cell mediated immune response against the transfected tumor cells. In addition, tumor cells which lack MHC class I or MHC class II molecules, or which fail to reexpress sufficient amounts of MHC class I or MHC class II molecules, can be transfected with nucleic acid encoding all or a portion of (*e.g.*, a cytoplasmic-domain truncated portion) of an MHC class I  $\alpha$  chain protein and  $\beta_2$  microglobulin protein or an MHC class II  $\alpha$  chain protein and an MHC class II  $\beta$  chain protein to thereby express MHC class I or MHC class II proteins on the cell surface. Expression of the appropriate class I or class II MHC in conjunction with a peptide having the activity of a B lymphocyte antigen (*e.g.*, B7-1, B7-2, B7-3) induces a T cell mediated immune response against the transfected tumor cell. Optionally, a gene encoding an antisense construct which blocks expression of an MHC class II associated protein, such as the invariant chain, can also be cotransfected with a DNA encoding a peptide having the activity of a B lymphocyte antigen to promote presentation of tumor associated antigens and induce tumor specific immunity. Thus, the induction of a T cell mediated immune response in a human subject may be sufficient to overcome tumor-specific tolerance in the subject.

The activity of a protein or a cognate Therapeutic of the invention may, among other means, be measured by the following methods: Suitable assays for thymocyte or splenocyte cytotoxicity include, without limitation, those described In: CURRENT PROTOCOLS IN

IMMUNOLOGY. Coligan *et al.*, eds. Greene Publishing Associates and Wiley-Interscience (Chapter 3, Chapter 7); Herrmann *et al.*, *Proc Natl Acad Sci USA* 78:2488-2492, 1981; Herrmann *et al.*, *J Immunol* 128:1968-1974, 1982; Handa *et al.*, *J Immunol* 20:1564-1572, 1985; Takai *et al.*, *J Immunol* 137:3494-3500, 1986; Takai *et al.*, *J Immunol* 140:508-512, 1988; Herrmann *et al.*, *Proc Natl Acad Sci USA* 78:2488-2492, 1981; Herrmann *et al.*, *J Immunol* 128:1968-1974, 1982; Handa *et al.*, *J Immunol* 18:1564-1572, 1985; Takai *et al.*, *J Immunol* 137:3494-3500, 1986; Bowman *et al.*, *J Virology* 61:1992-1998; Takai *et al.*, *J Immunol* 140:508-512, 1988; Bertagnolli *et al.*, *Cell Immunol* 133:327-341, 1991; Brown *et al.*, *J Immunol* 153:3079-3092, 1994.

Assays for T-cell-dependent immunoglobulin responses and isotype switching (which will identify, among others, proteins that modulate T-cell dependent antibody responses and that affect Th1/Th2 profiles) include, without limitation, those described in: Maliszewski, *J Immunol* 144:3028-3033, 1990; and Mond and Brunswick In: CURRENT PROTOCOLS IN IMMUNOLOGY. Coligan *et al.*, (eds.) Vol 1 pp. 3.8.1-3.8.16, John Wiley and Sons, Toronto 1994.

Mixed lymphocyte reaction (MLR) assays (which will identify, among others, proteins that generate predominantly Th1 and CTL responses) include, without limitation, those described in: CURRENT PROTOCOLS IN IMMUNOLOGY. Coligan *et al.*, eds. Greene Publishing Associates and Wiley-Interscience (Chapter 3, Chapter 7); Takai *et al.*, *J Immunol* 137:3494-3500, 1986; Takai *et al.*, *J Immunol* 140:508-512, 1988; Bertagnolli *et al.*, *J Immunol* 149:3778-3783, 1992.

Dendritic cell-dependent assays (which will identify, among others, proteins expressed by dendritic cells that activate naive T-cells) include, without limitation, those described in: Guery *et al.*, *J Immunol* 134:536-544, 1995; Inaba *et al.*, *J Exp Med* 173:549-559, 1991; Macatonia *et al.*, *J Immunol* 154:5071-5079, 1995; Porgador *et al.*, *J Exp Med* 182:255-260, 1995; Nair *et al.*, *J Virol* 67:4062-4069, 1993; Huang *et al.*, *Science* 264:961-965, 1994; Macatonia *et al.*, *J Exp Med* 169:1255-1264, 1989; Bhardwaj *et al.*, *J Clin Investig* 94:797-807, 1994; and Inaba *et al.*, *J Exp Med* 172:631-640, 1990.

Assays for lymphocyte survival/apoptosis (which will identify, among others, proteins that prevent apoptosis after superantigen induction and proteins that regulate lymphocyte homeostasis) include, without limitation, those described in: Darzynkiewicz *et al.*, *Cytometry* 13:795-808, 1992; Gorczyca *et al.*, *Leukemia* 7:659-670, 1993; Gorczyca *et al.*, *Cancer Res* 53:1945-1951, 1993; Itoh *et al.*, *Cell* 66:233-243, 1991; Zacharchuk, *J Immunol* 145:4037-

4045, 1990; Zamai *et al.*, *Cytometry* 14:891-897, 1993; Gorczyca *et al.*, *Internat J Oncol* 1:639-648, 1992.

Assays for proteins that influence early steps of T-cell commitment and development include, without limitation, those described in: Antica *et al.*, *Blood* 84:111-117, 1994; Fine *et al.*, *Cell Immunol* 155: 111-122, 1994; Galy *et al.*, *Blood* 85:2770-2778, 1995; Toki *et al.*, *Proc Nat Acad Sci USA* 88:7548-7551, 1991.

### **Tissue Growth Activity**

A FCTR<sub>X</sub> protein or a cognate Therapeutic of the present invention also may have utility in compositions used for bone, cartilage, tendon, ligament and/or nerve tissue growth or regeneration, as well as for wound healing and tissue repair and replacement, and in the treatment of burns, incisions and ulcers.

A protein or a cognate Therapeutic of the present invention, which induces cartilage and/or bone growth in circumstances where bone is not normally formed, has application in the healing of bone fractures and cartilage damage or defects in humans and other animals. Such a preparation employing a protein of the invention may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. De novo bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery.

A protein or a cognate Therapeutic of this invention may also be used in the treatment of periodontal disease, and in other tooth repair processes. Such agents may provide an environment to attract bone-forming cells, stimulate growth of bone-forming cells or induce differentiation of progenitors of bone-forming cells. A protein of the invention may also be useful in the treatment of osteoporosis or osteoarthritis, such as through stimulation of bone and/or cartilage repair or by blocking inflammation or processes of tissue destruction (collagenase activity, osteoclast activity, etc.) mediated by inflammatory processes.

Another category of tissue regeneration activity that may be attributable to the protein of the present invention is tendon/ligament formation. A protein of the present invention, which induces tendon/ligament-like tissue or other tissue formation in circumstances where such tissue is not normally formed, has application in the healing of tendon or ligament tears, deformities and other tendon or ligament defects in humans and other animals. Such a preparation employing a tendon/ligament-like tissue inducing protein may have prophylactic use in preventing damage to tendon or ligament tissue, as well as use in the improved fixation of tendon or ligament to bone or other tissues, and in repairing defects to tendon or ligament

tissue. De novo tendon/ligament-like tissue formation induced by a composition of the present invention contributes to the repair of congenital, trauma induced, or other tendon or ligament defects of other origin, and is also useful in cosmetic plastic surgery for attachment or repair of tendons or ligaments. The compositions of the present invention may provide an environment to attract tendon- or ligament-forming cells, stimulate growth of tendon- or ligament-forming cells, induce differentiation of progenitors of tendon- or ligament-forming cells, or induce growth of tendon/ligament cells or progenitors *ex vivo* for return *in vivo* to effect tissue repair. The compositions of the invention may also be useful in the treatment of tendonitis, carpal tunnel syndrome and other tendon or ligament defects. The compositions may also include an appropriate matrix and/or sequestering agent as a carrier as is well known in the art.

A protein or a cognate Therapeutic of the present invention may also be useful for proliferation of neural cells and for regeneration of nerve and brain tissue, *i.e.* for the treatment of central and peripheral nervous system diseases and neuropathies, as well as mechanical and traumatic disorders, which involve degeneration, death or trauma to neural cells or nerve tissue. More specifically, a protein may be used in the treatment of diseases of the peripheral nervous system, such as peripheral nerve injuries, peripheral neuropathy and localized neuropathies, and central nervous system diseases, such as Alzheimer's, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome. Further conditions which may be treated in accordance with the present invention include mechanical and traumatic disorders, such as spinal cord disorders, head trauma and cerebrovascular diseases such as stroke. Peripheral neuropathies resulting from chemotherapy or other medical therapies may also be treatable using a protein of the invention.

Proteins of the invention may also be useful to promote better or faster closure of non-healing wounds, including without limitation pressure ulcers, ulcers associated with vascular insufficiency, surgical and traumatic wounds, and the like.

It is expected that a protein of the present invention may also exhibit activity for generation or regeneration of other tissues, such as organs (including, for example, pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac) and vascular (including vascular endothelium) tissue, or for promoting the growth of cells comprising such tissues. Part of the desired effects may be by inhibition or modulation of fibrotic scarring to allow normal tissue to regenerate. A protein of the invention may also exhibit angiogenic activity.

A protein of the present invention may also be useful for gut protection or regeneration and treatment of lung or liver fibrosis, reperfusion injury in various tissues, and conditions resulting from systemic cytokine damage.

A protein of the present invention may also be useful for promoting or inhibiting differentiation of tissues described above from precursor tissues or cells; or for inhibiting the growth of tissues described above.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for tissue generation activity include, without limitation, those described in: International Patent Publication No. WO95/16035 (bone, cartilage, tendon); International Patent Publication No. WO95/05846 (nerve, neuronal); International Patent Publication No. WO91/07491 (skin, endothelium).

Assays for wound healing activity include, without limitation, those described in: Winter, EPIDERMAL WOUND HEALING, pp. 71-112 (Maibach and Rovee, eds.), Year Book Medical Publishers, Inc., Chicago, as modified by Eaglstein and Menz, *J. Invest. Dermatol* 71:382-84 (1978).

### **Receptor/Ligand Activity**

A protein or a cognate Therapeutic of the present invention may also demonstrate activity as receptors, receptor ligands or inhibitors or agonists of receptor/ligand interactions. Examples of such receptors and ligands include, without limitation, cytokine receptors and their ligands, receptor kinases and their ligands, receptor phosphatases and their ligands, receptors involved in cell—cell interactions and their ligands (including without limitation, cellular adhesion molecules (such as selectins, integrins and their ligands) and receptor/ligand pairs involved in antigen presentation, antigen recognition and development of cellular and humoral immune responses). Receptors and ligands are also useful for screening of potential peptide or small molecule inhibitors of the relevant receptor/ligand interaction. A protein of the present invention (including, without limitation, fragments of receptors and ligands) may themselves be useful as inhibitors of receptor/ligand interactions.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for receptor-ligand activity include without limitation those described in: CURRENT PROTOCOLS IN IMMUNOLOGY, Ed by Coligan, *et al.*, Greene Publishing Associates and Wiley-Interscience (Chapter 7.28, Measurement of Cellular Adhesion under static conditions 7.28.1-7.28.22), Takai *et al.*, *Proc Natl Acad Sci USA* 84:6864-6868, 1987;

Bierer *et al.*, *J. Exp. Med.* 168:1145-1156, 1988; Rosenstein *et al.*, *J. Exp. Med.* 169:149-160 1989; Stoltenborg *et al.*, *J Immunol Methods* 175:59-68, 1994; Stitt *et al.*, *Cell* 80:661-670, 1995.

### **Anti-Inflammatory Activity**

Proteins or cognate Therapeutics of the present invention may also exhibit anti-inflammatory activity. The anti-inflammatory activity may be achieved by providing a stimulus to cells involved in the inflammatory response, by inhibiting or promoting cell-cell interactions (such as, for example, cell adhesion), by inhibiting or promoting chemotaxis of cells involved in the inflammatory process, inhibiting or promoting cell extravasation, or by stimulating or suppressing production of other factors which more directly inhibit or promote an inflammatory response. Proteins exhibiting such activities can be used to treat inflammatory conditions including chronic or acute conditions), including without limitation inflammation associated with infection (such as septic shock, sepsis or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine-induced lung injury, inflammatory bowel disease, Crohn's disease or resulting from over production of cytokines such as TNF or IL-1. Proteins of the invention may also be useful to treat anaphylaxis and hypersensitivity to an antigenic substance or material.

### **Tumor Inhibition Activity**

In addition to the activities described above for immunological treatment or prevention of tumors, a protein of the invention may exhibit other anti-tumor activities. A protein may inhibit tumor growth directly or indirectly (such as, for example, via ADCC). A protein may exhibit its tumor inhibitory activity by acting on tumor tissue or tumor precursor tissue, by inhibiting formation of tissues necessary to support tumor growth (such as, for example, by inhibiting angiogenesis), by causing production of other factors, agents or cell types which inhibit tumor growth, or by suppressing, eliminating or inhibiting factors, agents or cell types which promote tumor growth.

### **Other Activities**

A protein or a cognate Therapeutic of the invention may also exhibit one or more of the following additional activities or effects: inhibiting the growth, infection or function of, or killing, infectious agents, including, without limitation, bacteria, viruses, fungi and other parasites; effecting (suppressing or enhancing) bodily characteristics, including, without limitation, height, weight, hair color, eye color, skin, fat to lean ratio or other tissue

pigmentation, or organ or body part size or shape (such as, for example, breast augmentation or diminution, change in bone form or shape); effecting biorhythms or circadian cycles or rhythms; effecting the fertility of male or female subjects; effecting the metabolism, catabolism, anabolism, processing, utilization, storage or elimination of dietary fat, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional factors or component(s); effecting behavioral characteristics, including, without limitation, appetite, libido, stress, cognition (including cognitive disorders), depression (including depressive disorders) and violent behaviors; providing analgesic effects or other pain reducing effects; promoting differentiation and growth of embryonic stem cells in lineages other than hematopoietic lineages; hormonal or endocrine activity; in the case of enzymes, correcting deficiencies of the enzyme and treating deficiency-related diseases; treatment of hyperproliferative disorders (such as, for example, psoriasis); immunoglobulin-like activity (such as, for example, the ability to bind antigens or complement); and the ability to act as an antigen in a vaccine composition to raise an immune response against such protein or another material or entity which is cross-reactive with such protein.

Neural disorders in general include Parkinson's disease, Alzheimer's disease, Huntington's disease, multiple sclerosis, amyotrophic lateral sclerosis (ALS), peripheral neuropathy, tumors of the nervous system, exposure to neurotoxins, acute brain injury, peripheral nerve trauma or injury, and other neuropathies, epilepsy, and/or tremors.

The invention will be further illustrated in the following non-limiting examples.

## EXAMPLES

### **Example 1. Method of Identifying the FCTR1 Nucleic Acid Encoding the Protein Resembling Neurite Outgrowth-Promoting Protein.**

A novel nucleic acid was identified by TblastN running the FCTR1 sequence (SEQ ID NO:1) against the Genomic Daily Files made available by GenBank. The FCTR1 nucleic acid was further analyzed by a sequence scanning software program to determine selection of exons and coding frames. These were further modified by means of similarities using BLAST searches. The sequence was then manually corrected for apparent inconsistencies, thereby obtaining the sequence for the full-length protein.

**Results.** A nucleic acid sequence originating from chromosome 11, in a region extending from nucleotide 77601 to nucleotide 79062 of the genomic clone in a negative reading frame, was identified in this work. The novel nucleic acid of 434 nucleotides



(designated Acc. No. AC009647-A) encoding a novel protein resembling neurite outgrowth-promoting protein is shown in Table 1A. An open reading frame was identified beginning with an ATG initiation codon at nucleotides 1-3 and ending with a TAG codon at nucleotides 430-432. A putative untranslated region following the termination codon is underlined in Table 1A, and the start and stop codons are in bold letters. The encoded protein having 143 amino acid residues is presented using the one-letter code in Table 1B.

In a search of sequence databases, it was found, for example, that the nucleic acid sequence has 426 of 434 bases (98%) identical to a human mRNA for a neurite outgrowth-promoting protein (GENBANK-ID: HSNOPMR|acc:X55110) (Table 1C). The expected probability of this match occurring by chance in the given database is  $1.2e-88$ , thus showing the very high probability that FCTR1 is a close homolog to human neurite outgrowth-promoting protein. The full amino acid sequence of the protein of the invention was found to have 137 of 143 amino acid residues (95 %) identical to, and 140 of 143 residues (97 %) positive with, a 143 amino acid residue human midkine precursor (neurite outgrowth-promoting protein) (ptr: SWISSPROT-ACC:P21741) (Table 1D). The expected probability of this match occurring by chance in the given database is  $4.1e-75$ , thus showing the very high probability that FCTR1 is a close homolog to this region of the human midkine precursor.

A multiple sequence alignment is given in Table 1E, with the FCTR1 polypeptide being shown on line 2, in a ClustalW analysis. The FCTR1 polypeptide is highly related to neurite outgrowth-promoting protein and other related protein sequences.

#### **Example 2. Method of Identifying the FCTR2 Nucleic Acid Encoding the Protein Resembling Sperm Antigen He-2.**

A novel nucleic acid was identified by TblastN running the FCTR2 sequence (SEQ ID NO:3) against the against Genomic Daily Files made publicly available by GenBank. The positive genomic clone was further analyzed by a sequence scanning software program to determine selection of exons and coding frames of FCTR2. Exons predicted by the program were then modified using Blast homologies with manual corrections to obtain a full-length protein of SEQ ID NO:4.

#### **Example 3. Method of Identifying the FCTR3 and FCTR4 Nucleic Acids Encoding the Polypeptides Resembling Somatotropin.**

A novel nucleic acid was identified by TblastN running the FCTR3 and FCTR4 sequences against the against Genomic Daily Files made publicly available by GenBank. Search results identified the 3' end of the FCTR3 protein. The contig sequence was further

extended by Blast homologies with various expressed sequence tags (EST's) to get the complete protein and then manually corrected for flaws. The nucleic acids were further analyzed by a sequence scanning software program to determine selection of exons and coding frames.

For FCTR3 specifically, sequence 508 to 812 from genomic clone region 53593\_53898 was used. Manual manipulation included fixing a frame shift and removed a 'G' from position 53735 of the clone. The complete full length contig of SEQ ID NO:5 was derived from ESTs, including AA371553, AA371672, AA371594, and C16883 for sequential extensions.

For FCTR4, sequence 508 to 808 from genomic clone region 53597\_53898 was used. Manual manipulation included fixing a frame shift and removed a 'G' from position 53735 of the clone. The complete full length contig of SEQ ID NO:7 was derived from ESTs, including AA371553, AA371672, AA371594, and C16883 for sequential extensions.

**Example 4. Method of Identifying the FCTR5 Nucleic Acid Encoding the Protein Resembling Neutrophil Activating Peptide Homolog.**

A novel nucleic acid was identified by TblastN running the FCTR5 sequence against the Genomic Daily Files made publicly available by GenBank. The FCTR5 nucleic acid was further analyzed by a sequence scanning software program to determine selection of exons and coding frames. Exons predicted by the program were then modified using Blast homologies with manual corrections to obtain a full-length protein.

Three genomic clones were found in a proprietary database with homology to SEQ ID NO:9 of FCTR5. In the first clone, the reverse frame of region 17126 to 17320 codes for residues 1 through 194. In the second clone, region 32479 to 32618 of the positive frame codes for residues 195 through 334. In the third clone, region 32744 to 32880 of the positive frame codes for residues 335 to 465. See, Table 5A.

**Example 5. Method of Identifying the FCTR6 Nucleic Acid Encoding the Protein Resembling Neutrophil Activating Peptide Homolog.**

A novel nucleic acid was identified by TblastN running the FCTR6 sequence against the Genomic Daily Files made publicly available by GenBank. The FCTR6 nucleic acid was further analyzed by a sequence scanning software program to determine selection of exons and coding frames. Exons predicted by the program were then modified using Blast homologies with manual corrections to obtain a full-length protein.

A genomic clone was identified with regions of homology to SEQ ID NO:11 of FCTR6. Region 107559 to 107722 codes for residues 1 through 164, region 108015 to 108090 codes for residues 165 through 240, region 108275 to 108331 codes for residues 241 through 297, and region 108445 to 108610 codes for residues 298 to 463. See, Table 6A.

**Example 6. Radiation Hybrid Mapping Identifies the Chromosomal Location of the Clones of the Invention.**

Radiation hybrid mapping using human chromosome markers was carried out for many of the clones described in the present invention. The procedure used to obtain these results is analogous to methods known in the art. See, *e.g.*, Steen et al. (A High-Density Integrated Genetic Linkage and Radiation Hybrid Map of the Laboratory Rat, Genome Research 1999 (Published Online on May 21, 1999) Vol. 9, AP1-AP8, 1999). Detailed description of RH mapping are also known. See, *e.g.*, Cox et al. (1990) *Science* 250: 245-250; Boehnke et al. (1991) *Am J Hum Genet* 49: 1174-1188; and Walter et al. (1994) *Nat Genet* 7: 22-28.

A panel of 93 cell clones containing randomized radiation-induced human chromosomal fragments was screened in 96 well plates using PCR primers designed to identify the sought clones in a unique fashion. Table 8 provides the results obtained for three clones of the present invention, showing the markers straddling the gene of the invention, and the distance in cR separating them.

**Table 8**

Clone	FCTR #	Chromosome	Distance from Marker, cR
AC009647-A	1	11	
AF202031-A	2	8p23.1-p22	
AC013573	5	4	WI-5565, 0.0
AL133124	6	4	WI-5565, 0.0

Although AC013573 (FCTR5) and AL133124 (FCTR6) map to the same location on chromosome 4, namely, they both coincide with marker WI-5565, the details of their radiation hybrid map arrays differ. Thus, while they may be close neighbors along chromosome 4, they do not represent the same genetic information.

**Example 7. Quantitative expression analysis of FCTR4 clone AC013601\_B in various cells and tissues.**

The quantitative expression of various clones was assessed in about 41 normal and about 55 tumor samples by real time quantitative PCR (TaqMan<sup>®</sup>) performed on a Perkin-Elmer Biosystems ABI PRISM<sup>®</sup> 7700 Sequence Detection System.

First, 96 RNA samples were normalized to  $\beta$ -actin and GAPDH. RNA (~50 ng total or ~1 ng polyA+) was converted to cDNA using the TAQMAN® Reverse Transcription Reagents Kit (PE Biosystems, Foster City, CA; Catalog No. N808-0234) and random hexamers according to the manufacturer's protocol. Reactions were performed in 20  $\mu$ l and incubated for 30 min. at 48°C. cDNA (5  $\mu$ l) was then transferred to a separate plate for the TAQMAN® reaction using  $\beta$ -actin and GAPDH TAQMAN® Assay Reagents (PE Biosystems; Catalog Nos. 4310881E and 4310884E, respectively) and TAQMAN® universal PCR Master Mix (PE Biosystems; Catalog No. 4304447) according to the manufacturer's protocol. Reactions were performed in 25  $\mu$ l using the following parameters: 2 min. at 50°C; 10 min. at 95°C; 15 sec. at 95°C/1 min. at 60°C (40 cycles). Results were recorded as CT values (cycle at which a given sample crosses a threshold level of fluorescence) using a log scale, with the difference in RNA concentration between a given sample and the sample with the lowest CT value being represented as 2 to the power of delta CT. The percent relative expression is then obtained by taking the reciprocal of this RNA difference and multiplying by 100. The average CT values obtained for  $\beta$ -actin and GAPDH were used to normalize RNA samples. The RNA sample generating the highest CT value required no further diluting, while all other samples were diluted relative to this sample according to their  $\beta$ -actin /GAPDH average CT values.

Normalized RNA (5  $\mu$ l) was converted to cDNA and analyzed via TAQMAN® using One Step RT-PCR Master Mix Reagents (PE Biosystems; Catalog No. 4309169) and gene-specific primers according to the manufacturer's instructions. Probes and primers were designed for each assay according to Perkin Elmer Biosystem's *Primer Express* Software package (version I for Apple Computer's Macintosh Power PC) or a similar algorithm using the target sequence as input. Default settings were used for reaction conditions and the following parameters were set before selecting primers: primer concentration = 250 nM, primer melting temperature ( $T_m$ ) range = 58°-60° C, primer optimal  $T_m$  = 59° C, maximum primer difference = 2° C, probe does not have 5' G, probe  $T_m$  must be 10° C greater than primer  $T_m$ , amplicon size 75 bp to 100 bp. The probes and primers selected (see below, Table AB) were synthesized by SyntheGen (Houston, TX, USA). Probes were double purified by HPLC to remove uncoupled dye and evaluated by mass spectroscopy to verify coupling of reporter and quencher dyes to the 5' and 3' ends of the probe, respectively. Their final concentrations were: forward and reverse primers, 900 nM each, and probe, 200nM.

TaqMan oligo set Ag351 for the FCTR4 gene (*i.e.*, AC013601\_B) include the forward (19 nt), probe (32 nt) and reverse (23 nt) oligomers that start at residues 86, 106 and 581, respectively. Sequences for the oligos are shown below:

Forward 5'-CCGGACTGGGCAGATCTTC-3'

(SEQ ID NO:13)

Probe TET-5' -AGCAGACCTACAGCAAGTTCGACACAACTCA-3' -TAMRA (SEQ ID NO:14)

Reverse 5'-CTTGAGTAGTGCATCGTTGT-3'

(SEQ ID NO:15)

PCR conditions: Normalized RNA from each tissue and each cell line was spotted in each well of a 96 well PCR plate (Perkin Elmer Biosystems). PCR cocktails including two probes (SEQX-specific and another gene-specific probe multiplexed with the SEQX probe) were set up using 1X TaqMan™ PCR Master Mix for the PE Biosystems 7700, with 5 mM MgCl<sub>2</sub>, dNTPs (dA, G, C, U at 1:1:1:2 ratios), 0.25 U/ml AmpliTaq Gold™ (PE Biosystems), and 0.4 U/μl RNase inhibitor, and 0.25 U/μl reverse transcriptase. Reverse transcription was performed at 48° C for 30 minutes followed by amplification/PCR cycles as follows: 95° C 10 min, then 40 cycles of 95° C for 15 seconds, 60° C for 1 minute. The results are shown below in Table 9.

Table 9. TaqMan results for clone AC013601\_B (run tm529t\_ag351)

Tissue Name	Rel. Expr. %	Tissue Name	Rel. Expr. %
Endothelial cells	0.0	Kidney (fetal)	0.0
Endothelial cells (treated)	0.0	Renal ca. 786-0	0.0
Pancreas	0.0	Renal ca. A498	0.0
Pancreatic ca. CAPAN 2	0.0	Renal ca. RXF 393	0.0
Adipose	0.0	Renal ca. ACHN	0.0
Adrenal gland	0.0	Renal ca. UO-31	0.0
Thyroid	0.0	Renal ca. TK-10	0.0
Salivary gland	0.0	Liver	0.0
Pituitary gland	0.0	Liver (fetal)	0.0
Brain (fetal)	0.0	Liver ca. (hepatoblast) HepG2	0.0
Brain (whole)	0.0	Lung	0.0
Brain (amygdala)	0.0	Lung (fetal)	0.0
Brain (cerebellum)	0.0	Lung ca. (small cell) LX-1	0.0
Brain (hippocampus)	0.0	Lung ca. (small cell) NCI-H69	0.0
Brain (substantia nigra)	3.6	Lung ca. (s.cell var.) SHP-77	0.0
Brain (thalamus)	0.0	Lung ca. (large cell) NCI-H460	0.0
Brain (hypothalamus)	100.0	Lung ca. (non-sm. cell) A549	0.0
Spinal cord	0.0	Lung ca. (non-s.cell) NCI-H23	0.0
CNS ca. (glio/astro) U87-MG	0.0	Lung ca (non-s.cell) HOP-62	0.0
CNS ca. (glio/astro) U-118-MG	0.0	Lung ca. (non-s.cl) NCI-H522	0.0
CNS ca. (astro) SW1783	0.0	Lung ca. (squam.) SW 900	0.0
CNS ca.* (neuro; met ) SK-N-AS	0.0	Lung ca. (squam.) NCI-H596	0.0
CNS ca. (astro) SF-539	0.0	Mammary gland	0.0
CNS ca. (astro) SNB-75	0.0	Breast ca.* (pl. effusion) MCF-7	0.0

CNS ca. (glio) SNB-19	0.0	Breast ca.* (pl.ef) MDA-MB-231	0.0
CNS ca. (glio) U251	0.0	Breast ca.* (pl. effusion) T47D	0.0
CNS ca. (glio) SF-295	0.0	Breast ca. BT-549	0.0
Heart	0.1	Breast ca. MDA-N	0.0
Skeletal muscle	0.0	Ovary	0.0
Bone marrow	0.0	Ovarian ca. OVCAR-3	0.0
Thymus	0.0	Ovarian ca. OVCAR-4	0.0
Spleen	0.0	Ovarian ca. OVCAR-5	0.0
Lymph node	0.0	Ovarian ca. OVCAR-8	0.0
Colon (ascending)	0.0	Ovarian ca. IGROV-1	0.0
Stomach	0.0	Ovarian ca.* (ascites) SK-OV-3	0.0
Small intestine	0.0	Uterus	0.0
Colon ca. SW480	0.0	Placenta	36.8
Colon ca.* (SW480 met)SW620	0.0	Prostate	0.0
Colon ca. HT29	0.0	Prostate ca.* (bone met)PC-3	0.0
Colon ca. HCT-116	0.0	Testis	0.0
Colon ca. CaCo-2	0.0	Melanoma Hs688(A).T	0.0
Colon ca. HCT-15	0.0	Melanoma* (met) Hs688(B).T	0.0
Colon ca. HCC-2998	0.0	Melanoma UACC-62	0.0
Gastric ca.* (liver met) NCI-N87	0.0	Melanoma M14	0.0
Bladder	0.0	Melanoma LOX IMVI	0.0
Trachea	0.0	Melanoma* (met) SK-MEL-5	0.0
Kidney	0.0	Melanoma SK-MEL-28	0.0

In Table 9, the following abbreviations are used:

ca. = carcinoma,

\* = established from metastasis,

met = metastasis,

s cell var= small cell variant,

non-s = non-sm =non-small,

squam = squamous,

pl. eff = pl effusion = pleural effusion,

glio = glioma,

astro = astrocytoma, and

neuro = neuroblastoma.

The results presented in Table 9 show that there is high expression of clone AC013601\_B in hypothalamus and placenta, and moderate to low expression in substantia nigra. This result suggests that the gene or its gene product may potentially be useful use in therapeutic approaches for neurodegenerative diseases.

## EQUIVALENTS

From the foregoing detailed description of the specific embodiments of the invention, it should be apparent that particular novel compositions and methods involving nucleic acids, polypeptides, antibodies, detection and treatment have been described. Although these particular embodiments have been disclosed herein in detail, this has been done by way of example for purposes of illustration only, and is not intended to be limiting with respect to the scope of the appended claims that follow. In particular, it is contemplated by the inventors that various substitutions, alterations, and modifications may be made as a matter of routine for a person of ordinary skill in the art to the invention without departing from the spirit and scope of the invention as defined by the claims. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

**What is claimed is:**

1. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:
  - a) a mature form of the amino acid sequence given by SEQ ID NO: 2, 4, 6, 8, 10 or 12;
  - b) a variant of a mature form of the amino acid sequence given by SEQ ID NO: 2, 4, 6, 8, 10 or 12, wherein any amino acid in the mature form is changed to a different amino acid, provided that no more than 15% of the amino acid residues in the sequence of the mature form are so changed;
  - c) the amino acid sequence given by SEQ ID NO: 2, 4, 6, 8, 10 or 12;
  - d) a variant of the amino acid sequence given by SEQ ID NO: 2, 4, 6, 8, 10 or 12 wherein any amino acid specified in the chosen sequence is changed to a different amino acid, provided that no more than 15% of the amino acid residues in the sequence are so changed; and
  - e) a fragment of any of a) through d).
2. The polypeptide of claim 1 that is a naturally occurring allelic variant of the sequence given by SEQ ID NO: 2, 4, 6, 8, 10 or 12.
3. The polypeptide of claim 2, wherein the variant is the translation of a single nucleotide polymorphism.
4. The polypeptide of claim 1 that is a variant polypeptide described therein, wherein any amino acid specified in the chosen sequence is changed to provide a conservative substitution.
5. An isolated nucleic acid molecule comprising a nucleic acid sequence encoding a polypeptide comprising an amino acid sequence selected from the group consisting of:
  - a) a mature form of the amino acid sequence given SEQ ID NO: 2, 4, 6, 8, 10 or 12;
  - b) a variant of a mature form of the amino acid sequence given by SEQ ID NO: 2, 4, 6, 8, 10 or 12 wherein any amino acid in the mature form of the chosen sequence is



changed to a different amino acid, provided that no more than 15% of the amino acid residues in the sequence of the mature form are so changed;

- c) the amino acid sequence given by SEQ ID NO: 2, 4, 6, 8, 10 or 12;
  - d) a variant of the amino acid sequence given by SEQ ID NO: 2, 4, 6, 8, 10 or 12, in which any amino acid specified in the chosen sequence is changed to a different amino acid, provided that no more than 15% of the amino acid residues in the sequence are so changed;
  - e) a nucleic acid fragment encoding at least a portion of a polypeptide comprising the amino acid sequence given by SEQ ID NO: 2, 4, 6, 8, 10 or 12 or any variant of said polypeptide wherein any amino acid of the chosen sequence is changed to a different amino acid, provided that no more than 10% of the amino acid residues in the sequence are so changed; and
  - f) the complement of any of said nucleic acid molecules.
6. The nucleic acid molecule of claim 5, wherein the nucleic acid molecule comprises the nucleotide sequence of a naturally occurring allelic nucleic acid variant.
7. The nucleic acid molecule of claim 5 that encodes a variant polypeptide, wherein the variant polypeptide has the polypeptide sequence of a naturally occurring polypeptide variant.
8. The nucleic acid molecule of claim 5, wherein the nucleic acid molecule comprises a single nucleotide polymorphism encoding said variant polypeptide.
9. The nucleic acid molecule of claim 5, wherein said nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of
- a) the nucleotide sequence given by SEQ ID NO: 1, 3, 5, 7, 9 or 11;
  - b) a nucleotide sequence wherein one or more nucleotides in the nucleotide sequence given by SEQ ID NO: 1, 3, 5, 7, 9 or 11 is changed from that given by the chosen sequence to a different nucleotide provided that no more than 15% of the nucleotides are so changed;
  - c) a nucleic acid fragment of the sequence given by SEQ ID NO: 1, 3, 5, 7, 9 or 11; and
  - d) a nucleic acid fragment wherein one or more nucleotides in the nucleotide sequence given by SEQ ID NO: 1, 3, 5, 7, 9 or 11 is changed from that given by the chosen

sequence to a different nucleotide provided that no more than 15% of the nucleotides are so changed.

10. The nucleic acid molecule of claim 5, wherein said nucleic acid molecule hybridizes under stringent conditions to the nucleotide sequence given by SEQ ID NO: 1, 3, 5, 7, 9 or 11, or a complement of said nucleotide sequence.
11. The nucleic acid molecule of claim 5, wherein the nucleic acid molecule comprises a nucleotide sequence in which any nucleotide specified in the coding sequence of the chosen nucleotide sequence is changed from that given by the chosen sequence to a different nucleotide provided that no more than 15% of the nucleotides in the chosen coding sequence are so changed, an isolated second polynucleotide that is a complement of the first polynucleotide, or a fragment of any of them.
12. A vector comprising the nucleic acid molecule of claim 11.
13. The vector of claim 12, further comprising a promoter operably linked to said nucleic acid molecule.
14. A cell comprising the vector of claim 12.
15. An antibody that binds immunospecifically to the polypeptide of claim 1.
16. The antibody of claim 15, wherein said antibody is a monoclonal antibody.
17. The antibody of claim 15, wherein the antibody is a humanized antibody.
18. A method for determining the presence or amount of the polypeptide of claim 1 in a sample, the method comprising:
  - (a) providing said sample;
  - (b) introducing said sample to an antibody that binds immunospecifically to the polypeptide; and
  - (c) determining the presence or amount of antibody bound to said polypeptide, thereby determining the presence or amount of polypeptide in said sample.

19. A method for determining the presence or amount of the nucleic acid molecule of claim 5 in a sample, the method comprising:
- (a) providing said sample;
  - (b) introducing said sample to a probe that binds to said nucleic acid molecule; and
  - (c) determining the presence or amount of said probe bound to said nucleic acid molecule,
- thereby determining the presence or amount of the nucleic acid molecule in said sample.
20. A method of identifying an agent that binds to the polypeptide of claim 1, the method comprising:
- (a) introducing said polypeptide to said agent; and
  - (b) determining whether said agent binds to said polypeptide.
21. A method for identifying a potential therapeutic agent for use in treatment of a pathology, wherein the pathology is related to aberrant expression or aberrant physiological interactions of the polypeptide of claim 1, the method comprising:
- (a) providing a cell expressing the polypeptide of claim 1 and having a property or function ascribable to the polypeptide;
  - (b) contacting the cell with a composition comprising a candidate substance; and
  - (c) determining whether the substance alters the property or function ascribable to the polypeptide;
- whereby, if an alteration observed in the presence of the substance is not observed when the cell is contacted with a composition devoid of the substance, the substance is identified as a potential therapeutic agent.
22. A method for modulating the activity of the polypeptide of claim 1, the method comprising introducing a cell sample expressing the polypeptide of said claim with a compound that binds to said polypeptide in an amount sufficient to modulate the activity of the polypeptide.
23. A method of treating or preventing a pathology associated with the polypeptide of claim 1, said method comprising administering the polypeptide of claim 1 to a subject

in which such treatment or prevention is desired in an amount sufficient to treat or prevent said pathology in said subject.

24. The method of claim 23, wherein said subject is a human.
25. A method of treating or preventing a pathology associated with the polypeptide of claim 1, said method comprising administering to a subject in which such treatment or prevention is desired a FCTR<sub>X</sub> nucleic acid in an amount sufficient to treat or prevent said pathology in said subject.
26. The method of claim 25, wherein said subject is a human.
27. A method of treating or preventing a pathology associated with the polypeptide of claim 1, said method comprising administering to a subject in which such treatment or prevention is desired a FCTR<sub>X</sub> antibody in an amount sufficient to treat or prevent said pathology in said subject.
28. The method of claim 15, wherein the subject is a human.
29. A pharmaceutical composition comprising the polypeptide of claim 1 and a pharmaceutically acceptable carrier.
30. A pharmaceutical composition comprising the nucleic acid molecule of claim 5 and a pharmaceutically acceptable carrier.
31. A pharmaceutical composition comprising the antibody of claim 15 and a pharmaceutically acceptable carrier.
32. A kit comprising in one or more containers, the pharmaceutical composition of claim 29.
33. A kit comprising in one or more containers, the pharmaceutical composition of claim 30.

34. A kit comprising in one or more containers, the pharmaceutical composition of claim 31.
35. The use of a therapeutic in the manufacture of a medicament for treating a syndrome associated with a human disease, the disease selected from a pathology associated with the polypeptide of claim 1, wherein said therapeutic is the polypeptide of claim 1.
36. The use of a therapeutic in the manufacture of a medicament for treating a syndrome associated with a human disease, the disease selected from a pathology associated with the polypeptide of claim 1, wherein said therapeutic is a FCTR<sub>X</sub> nucleic acid.
37. The use of a therapeutic in the manufacture of a medicament for treating a syndrome associated with a human disease, the disease selected from a pathology associated with the polypeptide of claim 1, wherein said therapeutic is a FCTR<sub>X</sub> antibody.
38. A method for screening for a modulator of activity or of latency or predisposition to a pathology associated with the polypeptide of claim 1, said method comprising:
- a) administering a test compound to a test animal at increased risk for a pathology associated with the polypeptide of claim 1, wherein said test animal recombinantly expresses the polypeptide of claim 1;
  - b) measuring the activity of said polypeptide in said test animal after administering the compound of step (a); and
  - c) comparing the activity of said protein in said test animal with the activity of said polypeptide in a control animal not administered said polypeptide, wherein a change in the activity of said polypeptide in said test animal relative to said control animal indicates the test compound is a modulator of latency of, or predisposition to, a pathology associated with the polypeptide of claim 1.
39. The method of claim 38, wherein said test animal is a recombinant test animal that expresses a test protein transgene or expresses said transgene under the control of a promoter at an increased level relative to a wild-type test animal, and wherein said promoter is not the native gene promoter of said transgene.

40. A method for determining the presence of or predisposition to a disease associated with altered levels of the polypeptide of claim 1 in a first mammalian subject, the method comprising:
- measuring the level of expression of the polypeptide in a sample from the first mammalian subject; and
  - comparing the amount of said polypeptide in the sample of step (a) to the amount of the polypeptide present in a control sample from a second mammalian subject known not to have, or not to be predisposed to, said disease,
- wherein an alteration in the expression level of the polypeptide in the first subject as compared to the control sample indicates the presence of or predisposition to said disease.
41. A method for determining the presence of or predisposition to a disease associated with altered levels of the nucleic acid molecule of claim 5 in a first mammalian subject, the method comprising:
- measuring the amount of the nucleic acid in a sample from the first mammalian subject; and
  - comparing the amount of said nucleic acid in the sample of step (a) to the amount of the nucleic acid present in a control sample from a second mammalian subject known not to have or not be predisposed to, the disease;
- wherein an alteration in the level of the nucleic acid in the first subject as compared to the control sample indicates the presence of or predisposition to the disease.
42. A method of treating a pathological state in a mammal, the method comprising administering to the mammal a polypeptide in an amount that is sufficient to alleviate the pathological state, wherein the polypeptide is a polypeptide having an amino acid sequence at least 95% identical to a polypeptide comprising the amino acid sequence given by SEQ ID NO: 2, 4, 6, 8, 10 or 12 or a biologically active fragment thereof.
43. A method of treating a pathological state in a mammal, the method comprising administering to the mammal the antibody of claim 15 in an amount sufficient to alleviate the pathological state.

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